RELATIONSHIP BETWEEN BILIRUBIN AND

AUDITORY FUNCTION IN PREMATURE NEONATES

BY

Gabriel Anne Bargen

Submitted to the graduate degree program in Audiology
and the Graduate Faculty of the University of Kansas
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy.

Committee:

_____
Chairperson

_____

_____

_____

_____

Date approved:_____

The Dissertation Committee for Gabriel Anne Bargen certifies
that this is the approved version of the following dissertation:

RELATIONSHIP BETWEEN BILIRUBIN AND

AUDITORY FUNCTION IN PREMATURE NEONATES

Committee:

_____
Chairperson

_____

_____

_____

_____

Date approved:_____

To the four individuals that love me unconditionally,
Jason, Peyton and my parents

ABSTRACT

Research has shown hyperbilirubinemia in preterm infants is more prevalent and its course more protracted than in term neonates. High levels of bilirubin have been documented to be toxic to the central nervous system and may cause neurological impairments in newborns. Impairment of auditory function is the most consistent abnormality, especially in premature infants. Hyperbilirubinemia is a risk factor for sensorineural hearing loss according the Joint Committee on Infant Hearing. If premature infants are at a greater risk for hyperbilirubinemia it can be assumed that they will be at a greater risk of having auditory dysfunction caused, or contributed to, by hyperbilirubinemia.

Currently, national guidelines are needed which address when treatment options should be considered and implemented regarding bilirubin levels in premature infants. The need for these guidelines is a necessity, as preterm infants require much closer follow-up and more aggressive therapy than full term infants. The objective for this research project was to determine the relationship between bilirubin levels and auditory function in preterm infants. The auditory brainstem response (ABR) and distortion product otoacoustic emission (DPOAE) tests were prospectively investigated in premature infants while monitoring total serum bilirubin (TSB) levels.

Ten subjects with a mean gestational age of 31.5 weeks participated in the study. The mean peak TSB was 7.91 mg/dL. Based on the peak TSB to birth weight ratio all subjects were assigned to the control group. Spearman's correlation coefficient indicated no significant correlation between peak TSB levels and auditory function. This study provided baseline data for further research evaluating auditory function in preterm hyperbilirubinemic subjects.

TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER 1

INTRODUCTION

The Joint Committee on Infant Hearing (JCIH) (2007) identifies hyperbilirubinemia, at a serum level requiring exchange transfusion, as a risk factor for progressive or delayed-onset sensorineural hearing loss (SNHL). Impairment of auditory function is the most consistent abnormality that is associated with permanent damage from hyperbilirubinemia, especially in premature infants (Volpe, 2001). Over the past five decades, research has shown that hyperbilirubinemia in pre-term infants is more prevalent, more severe, and its course more protracted than in term neonates (Billings, Cole, & Lathe, 1954; Harris, 1961; Watchko, 2000). Unfortunately, a reliable protocol for the consideration and initiation of treatment for hyperbilirubinemia in premature neonates is lacking.

In 1994, the American Academy of Pediatrics (AAP) published practice parameters for the management of hyperbilirubinemia in healthy term newborns. These parameters addressed babies born at 37 weeks gestational age (GA) or more. Ten years later (AAP 2004), clinical practice guidelines were extended to include newborn infants born at 35 weeks GA or later. Neither the practice parameters from 1994 nor the clinical practice guidelines from 2004 address the specific levels at which bilirubin becomes toxic to premature infants born prior to 35 weeks GA. Currently, there are no nationally recognized guidelines for premature infants that address the bilirubin levels at which treatment options should be considered and/or implemented.

Bilirubin can be evaluated by measuring total serum bilirubin (TSB), bilirubin/albumin (B/A) ratio, and unbound bilirubin, with the gold standard being measurement of TSB. Several researchers have made recommendations on guidelines for treatment intervention using varying

levels of TSB or other form of measuring bilirubin that address low-birth weight and premature infants (Cashore, 2000; Newman & Maisels, 1992; Maisels, 1999; Maisels, 2001; Watchko, 2000). However, none of these suggestions has led to standards that are accepted nationally or internationally. Institutions within the United States and around the world use different values and measurements to decide when to initiate treatment for hyperbilirubinemia in the premature population (Bhutani & Johnson, 2004; Gartner, Herrarias, & Sebring, 1998; Hansen, 1996; Wennberg et al., 2006). The majority of health care providers for premature infants in the United States have been using the AAP 1994 practice parameters for hyperbilirubinemia management, the AAP 2004 clinical practice guidelines, and other suggestions from hyperbilirubinemia research to determine the level of TSB to initiate treatment of hyperbilirubinemia in the premature neonate population. The lack of guidelines for bilirubin regarding when to intervene in premature neonates indicates the need for data to precisely define sensitivity and specificity of either TSB or free bilirubin concentration in determining risk for acute bilirubin neurotoxicity or chronic sequelae (kernicterus) (Wennberg et al., 2006). The need for accurate bilirubin level guidelines for treatment implementation in premature infants is a necessity (Bhutani & Johnson, 2004; Wennberg et al., 2006).

Several studies have shown that the primary site of lesion in the auditory system in the presences of hyperbilirubinemia is in the central auditory pathways (Conlee & Shapiro, 1991; Dublin, 1951; Gerrard, 1952; Kelemen, 1956; Oysu, Aslan, Ulubil, & Baserer, 2002; Shapiro & Conlee, 1991; Shapiro & Hecox, 1988; Shapiro & Hecox, 1989). However, controversy regarding the site of lesion stems from other studies that also have indicated damage to the peripheral auditory structures at the level of the hair cells and the auditory (VIIIth) nerve (Chisin, Perlman, & Sohmer, 1979; Kaga, Kitazumi, & Kodama, 1979; Matkin & Carhart, 1966;

Nakamura et al., 1985). Thus, the assessment of hearing function in this population should include tests of both the peripheral and central auditory systems.

The auditory brainstem response (ABR) can be used with any infant, premature or full-term, to determine if bilirubin is affecting auditory function. Multiple investigators have reported that the ABR can vary as a function of bilirubin level and is a sensitive tool for detecting bilirubin-induced auditory toxicity (Chisin et al., 1979; Funato, Tamai, Shimada, & Nakamura, 1994; Kaga, Kitazumi, & Kodama, 1979; Nakamura, Takada, Shimabuku, Matsuo, Matsuo, and Negishi, 1985; Perlman, Fainmesser, Sohmer, Tamari, Wax, & Pevsmer, 1983). ABR measurements in infants born at or near term with TSB values of less than 20 mg/dl have shown prolonged absolute latencies and interwave intervals, with documented reversible damage to auditory function (Agrawal, Shukla, Misra, Kapoor, and Malik, 1998; Tan, Skurr, & Yip, 1992; Wong, Chen, & Wong, 2005). Non-reversible changes in auditory function in the form of prolonged absolute latencies and interwave intervals, absent ABR and/or significantly elevated ABR thresholds were found in children born at or near term with TSB values above 20 mg/dl (Madden, Rutter, Hilbert, Greinwald, & Choo, 2002; Shapiro, Rosen, & Dixon, 2002).

Otoacoustic emission (OAE) testing is an objective indirect measure of cochlear outer hair cell function. Similar to the ABR, OAEs can be measured in infants to assess auditory function separating normal from impaired ears (Gorga, Neely, Bergman, Beauchaine, Kaminski, Peters et al., 1993, 1997; Gorga, Norton, Sininger, Cone-Wesson, Folsom, Vohr et al., 2000; Norton, Widen, Gorga, Folsom, Sininger, Cone-Wesson et al., 2000). Evoked OAEs are byproducts of the normally functioning outer hair cells of the cochlea and they are pre-neural (Prieve & Fitzgerald, 2002). OAEs are usually absent in ears with SNHL that exceed 30 to 50 dB HL. One type of evoked OAEs is distortion product otoacoustic emissions (DPOAEs).

DPOAEs are intermodulation distortion tones that the cochlea generates in response to a close pair of stimulus tones with different frequencies, f_1 and f_2 ($f_2 > f_1$), and with levels, L_1 and L_2, that may be equal or different (Lonsbury-Martin & Martin, 2007). DPOAEs have been used in studies involving neonatal hyperbilirubinemia in attempts to clarify the main site of audiological lesion (Oysu et al., 2002; Stein, Tremblay, Pasternak, Banerjee, Lindemann, & Kraus, 1996). Stein et al. (1996) found DPOAEs to be present in seven of eight ears tested and ABR to be abnormal in all ears tested in four pre-term infants with elevated bilirubin requiring phototherapy and/or exchange transfusion. Oysu et al. (2002) found definite cochlear involvement based on absent DPOAEs and ABRs in 26 of 30 subjects who had neonatal hyperbilirubinemia as a single risk factor for SNHL according to their medical chart. The other four subjects had postsynaptic auditory damage based on present DPOAEs with absent ABRs.

Using both ABR and DPOAEs to evaluate auditory function could assist with determining where hyperbilirubinemia affects the auditory pathway. Additionally, multiple researchers recommend dual evaluation with ABR and OAE in hyperbilirubinemic newborns to ensure proper diagnosis (Oysu et al., 2002; Rhee, Park, and Jang, 1999; Stein et al., 1996). Paradoxical findings, like those associated with auditory neuropathy (AN), lead to significant risk of undiagnosed or misdiagnosed auditory dysfunction or inappropriate audiological intervention if only ABR or OAE testing is used when completing auditory evaluation of newborns with jaundice. Since the introduction of AN as a clinical entity by Starr, Picton, Sininger, Hood, and Berlin in 1996, case studies of children with hyperbilirubinemia who demonstrate audiological findings which support the definition of AN have been described in the literature (Stein et al., 1996; Deltenre, Mansbach, Bozet, Clercx, & Hecox, 1997; Simmons & Beauchaine, 2000). Research has found a correlation between hyperbilirubinemia and

prematurity indicating they are both significant risk factors for AN (Rance, Beer, Cone-Wesson, Shepherd, Dowell, King et al., 1999; Madden et al., 2002). The dual use of ABR and OAE to evaluate auditory function of hyperbilirubinemic newborns, especially with the premature population, is a necessity and therefore both measurements were used for this study.

Given the lack of data related to TSB levels and auditory function in pre-term infants, the long-term objective for this research project is to determine this relationship. Such information can provide criteria for initiating intervention aimed at ameliorating the effects of high bilirubin levels on auditory function in this population. The present study initiated this goal by prospectively investigating premature infants 28 to 34 weeks GA admitted to the University of Kansas Hospital NICU. Auditory function was evaluated using ABR and DPOAE testing while monitoring TSB levels in an attempt to determine the level at which TSB begins to affect the auditory system. The knowledge gained from monitoring auditory function and TSB levels over time, in turn, can be used as a guide for initiating treatment to potentially prevent permanent damage to auditory function associated with high TSB levels.

It is hypothesized that as peak TSB levels worsen the ABR will indicate an increase in absolute latency of wave III and/or wave V and interwave latency of III-V and/or I-V in premature neonates. It is also hypothesized that as peak TSB levels improve the ABR threshold will improve in premature neonates. Finally, it is hypothesized that DPOAE responses will be unaffected by the improvement or deterioration of peak TSB levels in premature neonates.

CHAPTER 2

REVIEW OF THE LITERATURE

The previous section summarized the general question this study addresses and stated the hypotheses to be tested. This section expands that discussion and provides detailed information about bilirubin and the relationship between hyperbilirubinemia, the auditory system, the Auditory Brainstem Response (ABR), Otoacoustic Emissions (OAE), and how premature infants differ from full-term infants with regard to hyperbilirubinemia. The relationship between hyperbilirubinemia and auditory neuropathy/ dys-synchrony is also discussed.

Bilirubin

Bilirubin is produced from the break down of aged or dysfunctional red blood cells (Hass, 1999). Under normal processes, red blood cells are broken down into heme and globin, then heme is further broken down into iron and biliverdin. Biliverdin is reduced into unconjugated bilirubin by the liver, spleen, and bone marrow and transported to the liver by binding to albumin in the blood stream. Unconjugated bilirubin is lipid soluble, water insoluble, and neurotoxic (Shapiro, 2003). Albumin releases the unconjugated bilirubin in the liver where it is bound by the protein uridine diphosphoglucuronosyl transferase (UDPGT) to a water-soluble, non-toxic glucuronide becoming conjugated bilirubin (also known as direct bilirubin) which can be excreted in bile (Maisels, 1999; Hass, 1999; Shapiro, 2003).

Neonatal hyperbilirubinemia results from excessive production of bilirubin and the limited ability to excrete it (Dennery, Seidman, & Stevenson, 2001; Hass, 1999; Kaplan, Muraca, Hammerman, Rubaltelli, Vilei, Vreman et al., 2002; Maisels & Kring, 2006). Dennery et al.

(2001) and Shapiro (2003) report that newborn infants have several factors that increase their risk of developing physiologic jaundice or high serum bilirubin concentrations in the first days of life. First, unconjugated bilirubin is not readily excreted in newborn infants and the ability to conjugate bilirubin is limited (Dennery et al., 2001). Infants, more so in the premature population, have immature UDPGT which causes an increase in unconjugated bilirubin. Second, newborn infants have red blood cells with a decreased life span causing an increase in hemoglobin which produces bilirubin at a higher rate than adults (Brouillard, 1974, Shapiro, 2003). The immature UDPGT and increased hemoglobin are responsible for physiologic jaundice of the neonate (Dennery et al., 2001; Shapiro, 2003).

Total serum bilirubin (TSB) is a combination of conjugated and unconjugated bilirubin, which in neonates consists almost completely of unconjugated bilirubin (Shapiro, 2003). The unconjugated bilirubin in neonates typically binds to protein, mainly albumin, in the blood and once the blood binding capacity is exceeded, the unconjugated bilirubin enters the brain, interstitial fluid, and cerebrospinal fluid by crossing over the blood-brain barrier (Bratlid, 1990; Shapiro, 2003). Unconjugated or unbound bilirubin that readily crosses the blood-brain barrier causes cellular injury by inhibiting mitochondrial enzymes and interfering with DNA synthesis which induces DNA-strand breakage and inhibits protein synthesis and phosphorylation (Chuniaud, Dcssantc, Chantoux, Blondeau, Francon, & Trivin, 1996). Bilirubin also inhibits the uptake of tyrosine, a marker of synaptic transmission (Amato, Kilguss, Gelardi, & Cashore, 1994). Additionally, bilirubin has been found to inhibit the function of N-methyl-D-aspartate-receptor ion channels which indicates bilirubin can interfere with neuroexcitatory signals and impair nerve conduction specifically in the auditory nerve (Bratlid, 1990; Hoffmann, Zanelli, Kubin, Mishra, & Delivoria-Papadopoulos, 1996).

Cellular damage from bilirubin can cause neuronal damage in the central nervous system (CNS) when levels of bilirubin become pathologic, known as bilirubin encephalopathy or kernicterus (AlOtaibi, Blaser, MacGregor, 2005; Amin, Ahlfors, Orlando, Dalzell, Merle, & Guillet, 2001; Dennery et al., 2001; Shapiro & Nakamura, 2001). Kernicterus is specifically yellow staining of the basal ganglia (Amin et al., 2001; Dennery et al., 2001; Hansen, 1994). According to Dennery et al. (2001), normal levels of peak TSB are from 5 to 6 mg/dL. Once levels reach 7 to 17 mg/dL, exaggerated physiologic jaundice or hyperbilirubinemia occurs and at peak TSB levels above 17 mg/dL pathologic jaundice can be identified (Dennery et al., 2001). Damage in the CNS includes pathologic lesions in the globus pallidus and subthalamic nucleus, auditory and oculomotor brainstem nuclei, cerebellum, and hippocampus (Dublin, 1951; Ahdab-Barmada & Moossy, 1984; Shapiro, 2003). The corresponding sequelae of excessive neonatal hyperbilirubinemia or kernicterus comprise a tetrad including athetoid cerebral palsy, dysfunction of the auditory system, impairment of upward gaze (hypotonia oculomotor disturbances), and dental enamel hypoplasia of primary teeth (Shapiro & Nakamura, 2001). Further detail of how hyperbilirubinemia damages the auditory system is provided later in this chapter.

Treatment of Hyperbilirubinemia

Current interventions make the severe sequelae of hyperbilirubinemia rare (Maisels & McDonagh, 2008). Exchange transfusions and phototherapy are the staples of intervention for the jaundiced newborn. The goal of therapy, regardless of technique used, is to lower the concentration of circulating bilirubin or keep it from increasing (Maisels & McDonagh, 2008).

Pharmacologic treatment options have been used and are described along with exchange transfusion and phototherapy in the following section.

Exchange Transfusion

Exchange transfusion was used to treat severe hyperbilirubinemia beginning in the 1940s (Allen, Diamond, & Watrous, 1949; Allen, Diamond, & Vaughan, 1950; Diamond, 1948; Diamond, Allen, & Thomas, 1951). The technique rapidly eliminates bilirubin and circulating antibodies that can increase bilirubin from circulation by removing blood from the infant and replacing it with similar amounts of red blood cells and plasma (Dennery et al., 2001). Dennery and colleagues (2001) reported that the procedure is repeated until twice the blood volume has been replaced.

Many complications of exchange transfusions have been reported, including thrombocytopenia, portalvein thrombosis, necrotizing enterocolitis, electrolyte imbalance, graft-versus-host disease, apnea, bradycardia, cyanosis, vasospasm, hypoxic-ischemic encephalopathy, acquired immunodeficiency syndrome, and infection (Jackson, 1997; Keenan, Novak, Sutherland, Bryla, & Fetterly, 1985; Lauer, Githens, Hayward, Conrad, Yanagihara, & Tubergen, 1982; Maisels & Newman, 1995; Wallgren, & Faxelius, 1974; Watchko, 2000). Death associated with exchange transfusion has been reported in approximately 3 in 1000 procedures (Hovi & Siimes, 1985; Jackson, 1997; Keenan, Novak, Sutherland, Bryla, & Fetterly, 1985). Because of these complications and the improvement of phototherapy, which is a less invasive treatment, the use of exchange transfusion has decreased (Gartner, Herrarias, & Sebring, 1998; Maisels, 2001). The decrease in the frequency with which exchange transfusions are used to treat hyperbilirubinemia includes its use with infants with birth weights less than 1500 grams.

O'Shea, Dillard, Klinepeter, & Goldstein, (1992) found only two infants underwent exchange transfusion in a cohort of 833 infants weighing less than 1500 grams at birth born in North Carolina between 1985 and 1989. Maisels (2001) reported no exchange transfusions were performed in 1213 live births of infants weighing less than 1500 grams between 1988 and 1997 at William Beaumont hospital in Michigan.

Watchko and Claassen (1994) completed a retrospective review of postmortem and clinical records to determine the current prevalence of kernicterus. They also examined the relationship between the occurrence of kernicterus and the infants "at risk" status for exchange transfusion criteria. The records of 81 infants who were less than 34 weeks GA were reviewed. All the study infants received phototherapy and four received exchange transfusions. Kernicterus was observed in 3 of the infants with peak TSB levels of 26 mg/dL, 11.3 mg/dL, and 18.5 mg/dL. Of the other 78 subjects, peak TSB levels ranged from 3.6 to 22.5 mg/dL and only three were treated with exchange transfusion. Watchko and Claassen (1994) stated that even when TSB levels rise above those previously thought to place the infant at risk, kernicterus is unlikely to occur.

Guidelines for the use of exchange transfusion vary depending on GA, birth weight, and medical condition of the infant. Pearlman, Gartner, Lee, Morecki, & Horoupian (1978) published a set of widely used exchange transfusion criteria based on birth weight and serum bilirubin concentration (mg/dL) as criteria for exchange transfusion. Based on Pearlman et al. data, for full-term neonates with no other health concerns, exchange transfusion is recommended at TSB concentrations between 25 and 29 mg/dL (Newman & Maisels, 1992). The AAP published clinical practice guidelines in 2004 for the management of hyperbilirubinemia in newborn infants of 35 or more weeks GA. This document based the need for exchange

transfusion on Ahlfors' (1994) study using a bilirubin/albumin (B/A) ratio. According to Ahlfors (1994), exchange transfusion is recommended for infants of 35 or more weeks GA with no other health concerns at a B/A ratio of 7.2 mg/dL.

The guidelines for consideration and initiation of treatment with exchange transfusion vary as previously indicated. Additionally, these guidelines do not include pre-term infants. Physicians use unverified research on pre-term infants and estimate from the full-term infant guidelines to determine if exchange transfusion is needed when treating hyperbilirubinemic premature infants. Research based guidelines are needed in order to determine if exchange transfusion is the best course of treatment for pre-term infants especially given the complications associated with to use of this treatment.

Phototherapy

Phototherapy has been used to treat hyperbilirubinemia since its introduction in 1958 by Cremer, Perryman, & Richards. Phototherapy uses light energy to change the shape and structure of bilirubin, converting it to molecules that can be excreted even when normal conjugation is deficient (Lightner & McDonagh, 1984). There is no standardized method for delivering phototherapy according to the AAP (2004). Commonly used phototherapy units contain daylight, cool white, blue or "special blue" fluorescent tubes, tungsten-halogen lamps, or high-intensity gallium nitride light-emitting diodes and can be delivered via free-standing lamps or as part of a radiant warming device (AAP, 2004; Dennery et al., 2001; Seidman, Moise, Ergaz, Laor, Vreman, Stevenson et al., 2000; Vreman, Wong, Stevenson, Route, Reader, Fejer et al., 1998). According to Dennery et al. (2001), fluorescent white light is the most common form of phototherapy.

The spectral irradiance or dose delivered to the infant depends on the distance of the light source from the infant and the power of the light (Maisels, 1996; Lucey, Ferriero, & Hewitt, 1968). According to Maisels (1996) and Lucey et al. (1968), the lights should be placed as close to the infant as possible. The infant should also have as much skin exposed as possible to allow exposure of maximum surface area (Dennery et al., 2001). Phototherapy can be provided above and below the infant to increase exposure (Garg, Prasad, & Hifzi, 1995; Tan, 1991).

Many experts have provided guidelines for the use of phototherapy based on birth weight, GA, and different measurements of bilirubin (i.e., TSB vs B/A ratio) (Ahlfors, 1994; Ives, 1999; Maisels, 1999). According to the AAP (1994), phototherapy is generally recommended for an infant of 35 or more weeks GA with no other health concerns at 25 to 48 hours of life if the total bilirubin concentration reaches 15 mg/dL; 18 mg/dL at 49 to 72 hours; and 20 mg/dL at 72 hours or more.

Hulzebos, Van Imhoff, Bos, Ahlfors, Verkade, and Dijk (2008) published standard risk and high risk TSB level treatment thresholds for infants based on birth weight. The treatment thresholds were adapted from Ahlfors (1994) and Maisels & Watchko (2003) data regarding criteria for exchange transfusion in low birth weight newborns with elevated bilirubin. Hulzebos et al. (2008) based the treatment thresholds on infants with a GA less than 32 weeks who did not have chromosomal or syndromal abnormalities. The phototherapy treatment thresholds of TSB (μmol/L) and B/A ratio (μmol/g) for groups based on birth weight developed by Hulzebos et al. (2008) were used in this study to assign subjects into the control group (standard risk) or the study group (increased risk).

Bhutani et al. (1999) looked at the hour-specific TSB levels of 2840 healthy term or near-term infants to determine the accuracy of TSB levels prior to discharge as a predictor of

developing hyperbilirubinemia. Near term was defined by birth weight ≥2000 grams for GA ≥36 weeks or birth weight ≥2500 grams for ≥35 weeks GA (Bhutani et al., 1999). Bhutani et al. charted TSB as a function of age in hours when the bilirubin test was completed and assigned the data to four risk zones ranging from high to low risk. Infants falling above the 95th percentile were considered to be in the High Risk Zone. They found 68 of the 172 term or near-term infants that were designated to the High Risk Zone continued to have subsequent significant hyperbilirubinemia (Bhutani et al., 1999). When compared to the 1994 AAP guidelines, these findings correlated well with the TSB levels at which treatment options should be considered and implemented at ≤24 hours and at ≥48 hours of age. Bhutani et al. did place the time frame between 24 to 48 hours at a greater risk at lower TSB levels than the AAP guidelines.

Phototherapy guidelines, including those recommended by the AAP, are not based on large prospective studies and may not apply to all infants (Dennery et al., 2001). The AAP (2004) specifically states that the guidelines are based on limited evidence and the levels used are approximations. The concentration and duration of exposure at which bilirubin is neurotoxic are not known and estimations cannot be generalized to all infants (Dennery et al., 2001). Premature infants, those who are sick, and those who have hemolytic disease are at greater risk for neurotoxic effects and the subsequent development of kernicterus (Billings, Cole, & Lathe, 1954; Gartner, Snyder, Chaban, & Bernstein, 1970; Maisels & Watchko, 2003; Watchko & Claassen, 1994).

Pharmacologic Therapy

Various metalloporphyrins, including Sn-mesoporphyrin (SnMP), a potent inhibitor of heme oxygenase, have been successfully used to decrease the production of bilirubin in neonates

(Reddy, Naundaswamy, Mehta, Petrova, & Hegyi, 2003; Martinez, Garcia, Otheguy, Drummond, & Kappas, 2001). Suresh, Martin, and Soll (2003) reported metalloporphyrins act by competitively inhibiting the enzyme microsomal heme oxygenase, the rate limiting enzyme in the catabolism of heme to bilirubin. In this manner, metalloporphyrins decrease the production of bilirubin. This is in contrast to all other current methods of therapy for unconjugated hyperbilirubinemia, which act by increasing the excretion of bilirubin after it is formed (Suresh, Martin, & Soll, 2003).

Reddy et al., (2003) reported on a case involving a 32 GA pre-term infant with severe hyperbilirubinemia. After 10 hours of intense phototherapy, levels of bilirubin remained high and based upon existing guidelines from Maisels (1999) an exchange transfusion was necessary. However, the blood type needed for the transfusion was not readily available so, once United States Food and Drug Administration (FDA) approval was received they substituted the use of exchange transfusion with a single injection of SnMP. The TSB levels reduced 10 hours after the SnMP injection and TSB levels did not elevate again. This case demonstrated a single-dose of SnMP avoids the need for an exchange transfusion (Reddy et al., 2003).

Martinez et al. (2001) completed a clinical trial with healthy neonates with GA between 38 to 41 weeks investigating the effect of SnMP. They found that the administration of a single dose of SnMP at the time when hyperbilirubinemia is becoming severe (15 to 18 mg/dL) entirely eliminated the need for phototherapy in the SnMP-treated group. No side effects were observed and the SnMP-treated group also had a significantly reduced length of time they were under clinical care for hyperbilirubinemia compared to the control group (Martinez et al., 2001).

Tin-mesoporphyrin and other metalloporphyrin drugs are not approved by the US FDA for treatment of hyperbilirubinemia (AAP, 2004). Suresh, Martin, and Soll (2003) reported

further studies evaluating safety and efficacy of metalloporphyrins are necessary before pharmacologic treatment of hyperbilirubinemia can be considered for routine use. They point out that larger sample sizes in controlled randomized trials are needed to rule out an increased risk of adverse events with SnMP and similar metalloporphyrin treatments. Additionally, the authors report the need for identification of reliable risk factors for severe hyperbilirubinemia and kernicterus in order to target high-risk neonates (Suresh, Martin, & Soll, 2003).

Initiation of Treatment for Hyperbilirubinemia

Methods currently used to measure bilirubin levels are not precise. Laboratory measurement of direct bilirubin values can vary widely between laboratories (AAP, 2004). Additionally, published normative values for TSB levels have a wide variance (AAP, 1994). Different suggestions using varying levels of TSB or other forms of measuring bilirubin in infants are used in the United States and globally (Bhutani & Johnson, 2004; Gartner, Herrarias, & Sebring, 1998; Hansen, 1996; Wennberg, Ahlfors, Bhutani, Johnson, & Shapiro, 2006). As recently as 2006, Wennberg et al. reported there are insufficient published data to precisely define sensitivity and specificity of either TSB or free bilirubin concentration in determining risk for acute bilirubin neurotoxicity or chronic sequelae (kernicterus). As indicated by the studies reviewed in this section, national guidelines for the premature population do not exist that address bilirubin levels at which treatment options should be considered and/or implemented. The need for accurate bilirubin level guidelines for treatment implementation in full-term infants, let alone premature infants, are a necessity (Bhutani & Johnson, 2004; Wennberg et al., 2006).

Hyperbilirubinemia in Premature Neonates

Kernicterus often occurs at lower bilirubin concentrations in premature newborns as compared with term newborns (Watchko & Maisels, 2003). Gartner et al. (1970) reported nine of fourteen very low birth weight, premature infants studied post mortem had signs of kernicterus with peak TSB levels of 9.4 to 15.6 mg/dL which is below the TSB levels where exchange transfusion is recommended (>20 mg/dL) for term infants (AAP, 1994). van de Bor, van Zeben-van der Aa, Verloove-Vanhorick, Brand, and Ruys (1989) investigated the relationship between maximal TSB concentration and neurodevelopmental outcome in infants born prematurely at the corrected age of two years. Of 831 children, the mean peak TSB level was 10.4 mg/dL with a range of 2-3 to 20.2 mg/dL. The mean peak TSB level for children with normal neurodevelopment was 10.3 mg/dL while the mean peak TSB level for those with a minor handicap was 10.7 mg/dL and for those with major handicap was 11.4 mg/dL (van de Bor et al., 1989). Similar to Gartner et al., these levels found by van de Bor et al. are clearly below the AAP (1994) exchange transfusion level recommendations.

Research has been completed to evaluate the diagnosis of kernicterus in premature infants with low levels of bilirubin. Ahdab-Barmada and Moossy (1984) completed a seven year retrospective study evaluating the brain at autopsy of premature infants who were diagnosed with kernicterus to clarify the differences in interpretation of yellow staining. Yellow staining of nuclei of the CNS is one identifier used to confirm kernicterus postmortem. Yellow staining of the CNS nuclei occurred in premature infants at lower TSB levels than in full term infants which Ahdab-Barmada and Moossy (1984) reported was a major diagnostic problem. Kernicterus occurred in 97 of 630 (15.4%) autopsied neonates and the level of peak TSB decreased as GA decreased (Ahdab-Barmada & Moossy, 1984). The authors found more pronounced yellow

discoloration of brain stem cranial nerve nuclei in the premature group and limited staining of cerebellar dentate nuclei and medullary olivary nuclei at lower levels of TSB in premature infants younger than 29 weeks GA (Ahdab-Barmada & Moossy, 1984).

Watchko and Maisels (2003) reported the major cause of damage from hyperbilirubinemia at lower TSB levels were exaggerated neonatal red blood cells, hepatic, and gastrointestinal immaturity in premature infants when compared to full term infants. Turkel, Miller, Guttenberg, Moynes, and Hodgman (1982) evaluated the clinical and pathologic implications of kernicterus by comparing the histologic changes in the brains of 64 infants whose clinical histories had previously been compared. They found, on autopsy, physical findings of bilirubin staining associated with kernicterus were found more frequently than when compared to the clinical diagnosis of kernicterus prior to death. Essentially, kernicterus could be diagnosed postmortem despite the infant not having the clinical diagnosis of kernicterus prior to death. Turkel et al. (1982) reported that postmortem diagnosis of kernicterus in infants that have low levels of bilirubin may be due to nonspecific microscopic damage causing kernicterus to potentially be over diagnosed postmortem in this population.

Low-birth weight infants are more vulnerable than term infants to bilirubin-mediated CNS injury (Cashore, 2000). Cashore (2000) reported the postnatal maturation of hepatic bilirubin uptake and conjugation may be slower in premature infants due to accelerated red cell breakdown and lingering hepatic immaturity. With the increasing survival of infants born prematurely, it is difficult to specify what circulating concentrations of indirect bilirubin are too high. Because of this, no single recommendation may serve the needs of all nurseries and their low-birth weight patients. Cashore (2000) stated the established protocols giving weight-related criteria for exchange transfusion in pre-term infants have been informally modified to include

weight-related criteria for the early initiation of phototherapy. These weight-related standards for treatment by either phototherapy or exchange transfusion have not been validated according to Cashore (2000). Criteria based on bilirubin, birth weight and gestation have not been established for extremely low-birth weight infants or infants who are premature (Cashore, 2000). Cashore (2000) provided recommendations for phototherapy and exchange transfusion for premature infants of low-birth weight but these are not nationally accepted.

Institutional variations in the levels of bilirubin at which phototherapy and exchange transfusions are initiated in jaundiced premature newborns indicate that the current management of hyperbilirubinemia in pre-term infants is not evidence-based (Hansen, 1996; AAP, 1994). Amin, Ahlfors, Orlando, Dalzell, Merle, and Guillet (2001) studied pre-term infants to determine if bilirubin-albumin (B:A) molar ratio (MR) and unbound bilirubin (UB) are useful in predicting bilirubin encephalopathy compared to the gold standard TSB measurement. Amin et al. (2001) used ABR on 5 of the first 7 days of life to assess the 126 infants for bilirubin encephalopathy. This study found UB to be a more sensitive predictor of bilirubin-induced auditory toxicity as evaluated by ABR changes than either B:A MR or TSB levels in pre-term newborns (Amin et al., 2001). Their findings were similar to Nakamura et al. (1985) study findings and to a study completed by Cashore and Oh (1982).

Bhutani and Johnson (2004) reported TSB concentrations were known to be poor predictors of bilirubin toxicity in the sick or pre-term infant. They reported a more appropriate predictor of neurotoxicity for this population to be a measure of unbound or "free" bilirubin, but no such tests had been validated or were widely used at the time the article was written. Instead, Bhutani and Johnson (2004) stated lower TSB thresholds for intervention in infants in NICUs are commonly used as defined by neonatologists in the field.

Measuring Auditory Function in the Presence of Hyperbilirubinemia

Both ABR and OAE measurements have been used to assess auditory function in individuals with hyperbilirubinemia. The use of both evaluation tools along with other objective and subjective tests can assist with determining the site of lesion in the auditory pathway. The section below reviews ABR and OAE testing and the studies that have used these methods to evaluate hyperbilirubin and auditory function of neonates.

ABR

The ABR is a series of scalp-recorded electrical potentials generated in the auditory nerve and brainstem during the first 10 to 20 msec after onset of a transient stimulus (Møller, 1994). The ABR tracing is a series of peaks and troughs representing far-field synchronous activity of the auditory nerve and auditory brainstem pathway (Stegeman, Van Oosterom, & Colon, 1987). ABR testing goes beyond the cochlea of the inner ear and assesses the lower auditory pathways. Generator sites for the various ABR components have been suggested by several studies. Wave I is generated in the distal portion of the nerve within the cochlea; wave II is generated in the proximal portion of the nerve, most likely at the junction between the nerve and the brainstem; wave III is generated within the cochlear nucleus, either from neuronal elements or from VIIIth nerve fibers within the structure (Møller, 1994). Møller, Jho, Yokota, and Jannetta (1995) found indications from ABR latency studies that wave IV is generated by structures close to the midline at the level of the superior olivary complex in the brainstem. Møller, Jannetta, and Jho (1994) suggested wave V may be generated by the lateral lemniscus as it leaves the cochlear nucleus.

The major measures of the ABR are the latency and amplitude of its peaks, and the differences (i.e., interwave intervals) between peaks I-III, III-V, and I-V (Don & Kwong, 2002). In addition, the threshold of the ABR is a good predictor for the behavioral threshold of hearing (Sininger & Cone-Wesson, 2002; Sininger, 1993). Given that the majority of previous research regarding ABR and hyperbilirubinemia utilizes latency and threshold measurements, this study also utilized latency and threshold. Therefore amplitude, although an important ABR measurement as indicated by previous ABR research, is not defined by this paper.

ABR peak latency is the time after stimulus onset that a given peak occurs (Don & Kwong, 2002). Latencies are used clinically as they are robust measures which are nearly unaffected by variations in electrode placement (Don & Kwong, 2002). ABR peak latencies are affected by the age of the patient and the level of stimulation (Don & Kwong, 2002; Ballachanda, Crawford, Ferraro, & Griffiths, 2004). As age increases from infant to adult peak latencies decrease (shorten) and as stimulus level increases peak latencies decrease (shorten). Essentially, the latencies of the ABR waveform components are longer in neonates compared with adults (Arnold, 2000; Ballachanda et al., 2004).

Premature infants will have even more prolonged ABR component latencies compared to full term infants and adults (Arnold, 2000; Ballachanda et al., 2004). Amin, Orlando, Dalzell, Merle, and Guillet (1999) determined morphological changes in ABRs during the first postnatal week of life in premature infants ≤32 weeks GA. They found the frequency of detection of waves improves over the first week of life. Additionally, absolute wave latencies and interwave intervals progressively decreased during the first postnatal week (Amin, 1999).

Gorga, Reiland, Beauchaine, Worthington, and Jesteadt (1987) evaluated ABR recordings from 585 babies having presumably normal hearing who graduated from a NICU.

The study group had conceptional ages (GA plus chronological age) from 33 weeks to 44 weeks. They found latencies, both absolute and interwave intervals, decreased as conceptional age increased. Rotteveel, de Graaf, Colon, Stegeman, and Yisco (1987) evaluated ABR recordings from 65 pre-term infants with conceptional ages from 25 weeks to 52 weeks. Similar to Gorga et al. (1987), they found latencies, both absolute and interwave intervals, decreased as conceptional age increased. Given the findings of these studies, ABR latencies for premature population should be compared to an appropriate set of age-dependent norms based on conceptional age.

The lowest level at which repeatable components of the ABR waveform can be detected provides information about the threshold of hearing (Arnold, 2000; Sininger & Cone-Wesson, 2002). The threshold of the ABR is a good predictor for hearing threshold as the threshold correlates with the threshold of hearing sensitivity. Specifically, the click threshold reportedly correlates best with hearing sensitivity between 1 and 4 kHz (Hyde, 1985; Jerger & Mauldin, 1978). According to Arnold (2000), normal hearing individuals along with those with conductive hearing loss have a click threshold approximately 10- to 20-dB higher than the best audiometric threshold. Those individuals with cochlear losses may have a difference of only 5 dB above the best behavioral threshold (Arnold, 2000).

ABR and Hyperbilirubinemia

The ABR has been proposed as an objective tool to evaluate acute bilirubin encephalopathy not only because it is technically feasible to use at the bedside but also because the auditory system is probably the most sensitive neural system to clinically-evident bilirubin injury (Johnston, Angara, Baumal, Hawke, Johnson, Keet et al., 1967; Chisin et al., 1979; Volpe, 2001). Multiple investigators have reported the use of ABR as a sensitive measure for detecting

bilirubin-induced auditory toxicity and have shown ABR to vary as a function of bilirubin (Chisin et al., 1979; Funato, Tamai, Shimada, & Nakamura, 1994; Kaga, Kitazumi, & Kodama, 1979; Nakamura et al., 1985; Perlman, Fainmesser, Sohmer, Tamari, Wax, & Pevsmer, 1983).

ABR measurements in children born at or near term with TSB values of less than 20 mg/dl have shown prolonged latencies, with documented reversible damage to auditory function (Agrawal, Shukla, Misra, Kapoor, and Malik, 1998; Tan, Skurr, & Yip, 1992; Wong, Chen, & Wong, 2005). Non-reversible changes in auditory function in the form of significantly elevated ABR thresholds were found in children born at or near term with TSB values above 20 mg/dl (Madden, Rutter, Hilbert, Greinwald, & Choo, 2002; Shapiro, Rosen, & Dixon, 2002).

Perlman et al. (1983) evaluated pathophysiologic changes in the auditory pathway during the period of hyperbilirubinemia in term infants by recording ABR when TSB levels were elevated and when TSB levels were reduced following treatment. They found a significant loss of waves IV and V and the ABR interwave intervals I-III and III-IV/V to be significantly prolonged in infants with increased bilirubin levels. Additionally, of 13 jaundiced subjects that had repeated recordings over a period of several days, nine showed apparent improvement in the ABR recordings with appearance of waves that were initially absent or by shortening of initially prolonged latencies. The changes noted in the loss of waves IV and V and the prolonged latencies indicated abnormal lower and upper brainstem functioning. As the subjects were free of pathologic processes other than hyperbilirubinemia, Perlman et al. state it is probable that the observed abnormal brainstem function was due to an acute toxic encephalopathy caused by the bilirubin. As the ABRs showed improvement when the TSB levels decreased back to a level of standard risk this statement was reinforced.

Tan, Skurr, and Yip (1992) assessed changes in ABR before, during and after phototherapy in 30 full-term hyperbilirubinemic neonates. Study subjects had significantly prolonged latency of wave V and interwave intervals III-V and I-V prior to and during phototherapy when compared to a control group. The differences in the latencies were not significant between the groups after phototherapy treatment was completed. Findings from this study supported previous research reporting phototherapy effectively reduces TSB values and by doing so reverses the effects of bilirubin on the ABR.

Vohr, Kapr, O-Dea, Darrow, Coll, Lester et al. (1990) evaluated 50 full-term infants with moderate hyperbilirubinemia (TSB of 10 to 20 mg/dL) and found the latency of brainstem auditory evoked responses was longer in infants with moderate TSB levels than in those with low TSB levels. They found the ABR abnormality to be more pronounced in infants with higher bilirubin concentrations.

When recording ABRs in neonates with hyperbilirubinemia the measurements that appear to have the most clinical relevance according to previous research are absolute latency of wave V and the interwave intervals III-V and I-V. The ABR latency difference in the premature population needs to be considered when evaluating ABR recordings in the presence of hyperbilirubinemia. It may be difficult to determine if latency differences between control group and study group subjects are due to conceptional age or effects from increased bilirubin. To elevate this problem, subjects from the same conceptional age groups should be compared based on the findings by Gorga et al. (1987) and Rotteveel et al. (1987).

DPOAEs

Kemp first described OAEs in 1978 as sounds generated in the inner ear that can be recorded in the ear canal. OAEs are believed to be the byproducts of the preneural mechanisms of the cochlear amplifier and to be linked to the normal functioning of the outer hair cells of the cochlea (Oysu et. al., 2002; Prieve & Fitzgerald, 2002). OAE testing is an objective indirect measure of cochlear function. OAEs are usually reduced in level or absent in ears with SNHL that exceed 30 to 50 dB HL (Gorga, Neely, Bergman, Beauchaine, Kaminski, Peters et al., 1993b; Gorga, Neely, Ohlrich, Hoover, Redner, & Peters, 1997; Gorga, Norton, Sininger, Cone-Wesson, Folsom, Vohr et al., 2000; Kemp, 1978; Prieve & Fitzgerald, 2002; Robinette, 1992; Stevens, 1988).

DPOAEs are intermodulation distortion products that undergo reverse transduction through the middle ear and are converted to acoustic energy that can be measured in the ear canal (Prieve & Fitzgerald, 2002). DPOAEs are a result of the nonlinear behaviors of the cochlea. The intermodulation distortion tones are generated by the cochlea in response to a close pair of stimulus tones. The stimulus tones have frequencies of the primaries, f_1 and f_2 ($f_2 > f_1$), and have levels of the primaries, L_1 and L_2, that may be equal or different (Lonsbury-Martin & Martin, 2007). DPOAEs occur at predictable frequencies that are mathematically related to the frequencies of the primaries which make them measurable using a narrowband filtering centered at the frequency of interest (Prieve & Fitzgerald, 2002).

Several studies have investigated the accuracy of DPOAEs to separate normal from impaired ears (Gorga et al., 1993b, 1997; Gorga, Norton, Sininger, Cone-Wesson, Folson, Vohr et al., 2000; Norton, Widen, Gorga, Folsom, Sininger, Cone-Wesson et al., 2000). These studies suggested DPOAEs accurately identify auditory status for middle and high frequencies, most

accurately from 2 to 4 kHz, but perform more poorly as the frequency decreases due to problems with noise below 2 kHz. DPOAEs perform well in the presence of minimal low frequency environmental noise (Prieve & Fitzgerald, 2002). DPOAE testing should be completed while patients are quiet or sleeping to reduce environmental noise.

Differences in DPOAEs have been found between the ears of full-term and premature infants. Smurzynski, Jung, Lafreniere, Kim, Kamath, Rowe et al. (1993) reported that approximately 20% of pre-term infant ears had OAEs greater than the 90th percentiles of full-term newborns, mostly in the 2.8- to 4-kHz region. Again in 1994, Smurzynski found pre-term infant ears to be greater than the 90th percentile of the full-term normal range in the 2.8- and 5.6-kHz bands (31%) and in the 4.0-kHz region (69%). Smurzynski et al. (1993) and Smurzynski (1994) also found the DPOAE level in premature infants to increase as the conceptional age increases especially in the 2.8 and 4.0 kHz bands. Smurzynski (1994) reported based on previous research by Keefe, Bulen, Arehart, and Burns (1993) that developmental changes occurring in the external and middle ear influence the signal transfer from the probe inserted in the ear canal to the cochlea take part in this age effected growth of the DPOAE level.

Abdala, Oba, and Ramanathan (2008) described and defined changes in the pre-term infant DP-gram through the first 6 months of postnatal life in order to provide normative guidelines to monitor infant hearing status. Abdala et al. (2008) found the infant DPOAE amplitude to be larger than adult at all ages tested which was similar to the findings of Brown (2000) and Smurzynski (1994). Abdala et al. (2008) found that baseline DPOAE levels of premature neonates are lower than those observed one month later by 3 to 5 dB. The DPOAE level increased during the pre-term period as a function of conceptional age. Infants born after a term-birth did not show this increase in DPOAE with age. Keefe and Abdala (2007) evaluated

the enlarged DPOAE input/output functions found in newborns to six months of age by looking at forward and reverse transfer function of the immature ear canal and middle ear components. They found DPOAE levels were larger in infants primarily due to the reverse middle ear transmittance level varied with ear canal area. This differed between term infants and adults by more than a factor of seven.

OAEs and Hyperbilirubinemia

OAE tests have been used in studies involving neonatal hyperbilirubinemia to attempt to clarify the main site of lesion in the auditory pathway. Additionally, OAE tests have been used in research of neonates with hyperbilirubinemia to assess the tools reliability in the presences of bilirubin. Rhee et al. (1999) used transient evoked OAEs to clarify the auditory pathway lesion site and to test the reliability of transient evoked OAEs (TEOAEs) in hearing screening of hyperbilirubinemic neonates. The results found the site of lesion in the auditory pathway caused by hyperbilirubinemia to be retrocochlear with the cochlea remaining intact. The authors suggested the TEOAEs to have limitations in evaluation of hearing in the neonates with hyperbilirubinemia as all eleven subjects passed the TEOAE screen but four had abnormal results with ABR thresholds above 40 dB nHL. Stein, Tremblay, Pasternak, Banerjee, Lindemann, and Kraus (1996) found hyperbilirubin to be a common characteristic in four patients they evaluated who were diagnosed with AN (present OAEs and absent or abnormal ABR). All four had elevated bilirubin levels warranting phototherapy treatment and one subject had an exchange transfusion. OAEs were found to be present indicating normal function of the cochlea and site of lesion to be retrocochlear. Stein et al. (1996) found DPOAEs to be present in seven of eight ears tested and ABR to be abnormal in all ears tested in four pre-term infants with

elevated bilirubin requiring phototherapy and/or exchange transfusion. Oysu and colleagues (2002) used DPOAEs and ABR to determine the incidence of cochlear impairment in a large group of patients with hyperbilirubinemia as a single risk factor for auditory dysfunction. They found four of 30 patients (13%) to have DPOAEs within the normal range in the presence of absent ABR waveforms and the rest of the patients to have definite cochlear involvement based on absent DPOAEs. Oysu et al. reported using only OAE testing or only ABR testing when completing auditory screening of newborns with jaundice carries a significant risk of undiagnosed or misdiagnosed auditory dysfunction or recommendation of inappropriate use of hearing aids. They recommended dual screening with ABR and OAE testing in hyperbilirubinemic newborns.

Auditory Damage from Hyperbilirubinemia

Impairment of auditory function is the most consistent abnormality that is associated with chronic post-kernicteric bilirubin encephalopathy, especially in premature infants (Volpe, 2001). Shapiro, Bhutani and Johnson (2006) reported seeing hyperbilirubinemia patients with impaired auditory function without athetosis or an associated movement disorder, the other problems associated with hyperbilirubinemia.

The nature and location of hearing loss contributed to by neonatal hyperbilirubinemia remain controversial and in most cases is not obvious (Billings & Kenna, 1999; Chisin et al., 1979; Oysu et al., 2002). This problem may relate to the comorbidity of hearing loss risk factors. Current research summarized in the following section points to different levels of the peripheral and central auditory pathways as the site of impairment. Use of the ABR in conjunction with OAE testing may help to define the site of impairment in infants with elevated TSB.

Animal Studies

Studies measuring ABRs on hyperbilirubinemic jj Gunn rats with deficient liver enzymes found functional abnormalities of the CNS (Shapiro & Hecox, 1988; Shapiro & Hecox, 1989). The ABRs of these hyperbilirubinemic jj Gun rats show prolongation of interwave intervals and the loss of all waves in severely affected animals similar to human studies (Conlee & Shapiro, 1991; Shapiro & Conlee, 1991; Shapiro & Hecox, 1988; Shapiro & Hecox, 1989). Shapiro and Nakamura (2001) completed autopsies of the hyperbilirubinemic jj Gunn rats and found damage to the auditory system which correlates with the known sites of bilirubin damage in the auditory brainstem pathways of humans specifically the brainstem ventral cochlear nucleus, lateral superior olivary nuclei, superior paraolivary nuclei, lateral lemnisci, the trapezoid bodies, and the cell bodies of the auditory nerve in the spiral ganglia of the cochlea. The inner ears of these animals were normal even with severely abnormal or absent ABRs (Shapiro & Nakamura, 2001). In 1993, Shapiro also reported ABR abnormalities found in hyperbilirubinemic jj Gunn rats have improved post treatment, similar to human studies.

Human Studies

Dublin (1951) was first to document that the main site of pathology in the presence of hyperbilirubinemia in humans is in the central auditory pathways. Autopsy studies by Haymaker, Margles, and Pentschew (1961), Dublin (1951), and Dublin (1976) of infants with classic kernicterus and one by Ahdab-Barmada and Moossy (1984) of premature infants who had low-bilirubin kernicterus showed central auditory pathology involving the brainstem auditory structures. Damaged brainstem auditory structures included dorsal and ventral cochlear nuclei, superior olivary complex and nuclei of the lateral lemniscus and inferior colliculus. Dublin

(1951) specifically noted the absence of significant abnormalities of the VIIIth nerve. Additional autopsy studies by Gerrard (1952) and Kelemen (1956) found no significant abnormalities of the inner ear structures.

Matkin and Carhart (1966) completed an audiological study on 22 subjects from eight to ten years of age who had a diagnosis of neonatal jaundice due to Rh incompatibility. The battery of audiological tests administered included conventional pure-tone threshold audiometry, monaural bifrequency loudness balancing test, short increment sensitivity index (SISI) test, the tone decay test, and the simultaneous binaural median-plane localization test to attempt to find the site of the auditory lesion. Results indicated lesions in the cochlear nuclei based on subjects not achieving binaural fusion during the simultaneous median-plane localization task. Responses from nineteen subjects suggested cochlear lesions on at least three of the tests completed. Additionally, the authors propose dual dysfunctions of the auditory system in both the cochlea and the central auditory pathways, but the existence of the central lesion might not be apparent or appeared as cochlear lesions due to the type of stimulus used by investigators (Matkin & Carhart, 1966).

Chisin et al. (1979) attempted to localize the auditory system lesion using conventional pure-tone threshold audiometry, ABR, and electrophysiological test measuring cochlear microphonic potential (CM). Thirteen patients with histories of neonatal hyperbilirubinemia and a positive diagnosis of sensorineural hearing loss took part in this study. Nine of the subjects had absent ABR and present CM. Results found functional evidence of auditory nerve damage with normal functioning hair cells in patients with hearing loss following neonatal hyperbilirubinemia (Chisin et al., 1979).

Nakamura et al. (1985) recorded ABR measurement before and after treatment to investigate the effects of bilirubin concentration in hyperbilirubinemic term infants. They found the absolute latencies of wave I and V to be prolonged in the study group and to improve following exchange transfusion; however, the interwave latencies did not change after treatment and the latencies were not significantly different between the control infants and hyperbilirubinemic infants. Nakamura et al. (1985) stated the lack of difference of the interwave latency of wave I-V between the groups indicated the auditory pathology to be outside the brainstem in either the cochlea or auditory nerve.

Kaga, Kitazumi, and Kodama (1979) evaluated ABRs and behavioral audiometry of 25 infants with kernicterus to determine the level of the lesion causing their hearing disorder. They reported 88% had ABR threshold elevation compared to age-matched normal subjects showed ABR abnormalities associated with peripheral hearing loss and 84% of these had behavioral threshold elevation. Abnormalities in the ABR included wave V threshold elevation, prolonged latency of wave I and V, and the absence of ABRs (Kaga et al., 1979). The authors stated that their results indicated at least some lesions which produce hearing disorders in kernicterus occur in the cochlea or auditory nerve.

Rhee, Park, and Jang (1999) evaluated auditory function of eleven patients with severe hyperbilirubinemia (mean peak TSB 29.2 ± 3.1 mg/dL) following exchange transfusion every three months up to one year by measuring TEOAEs and ABR threshold. TEOAEs were found to be normal in all patients while ABR responses were abnormal or absent in four patients. Of the four patients with abnormal ABRs, two showed improvement in auditory function, evident in improved ABR threshold responses at follow up tests. Rhee et al. (1999) found the hyperbilirubinemia-induced auditory dysfunction site of lesion to be retrocochlear.

Sheykholeslami and Kaga (2000) evaluated three patients with confirmed hearing loss using ABRs, OAEs, and traditional audiometry in attempts to localize the pathologic changes in auditory function in individuals who were diagnosed with severe hyperbilirubinemia as neonates. Their findings suggested some lesions which produced hearing loss in severe hyperbilirubinemia were in the cochlea, specifically the outer hair cells. As the peak TSB levels of the three patients were 16.2 mg/dL, 17.4 mg/dL, and 19 mg/dL, the authors also concluded that moderate elevation of TSB levels (<20 mg/dL) may contribute to the development of sensorineural hearing loss (Sheykholeslami & Kaga, 2000). This study is the only study I found to indicate damage to the outer hair cells from hyperbilirubinemia but it does add speculation to the auditory site of lesion from hyperbilirubinemia.

Oysu et al. (2002) attempted to determine the amount of cochlear involvement in 30 subjects (mean ± SD age of 32 ± 9.3 months) who had severe hyperbilirubinemia (mean ± SD peak TSB level of 24.4 ± 8.8 mg/dL in the neonatal period) as a single risk factor for sensorineural hearing loss from a group of 1,032 pediatric patients. Audiological tests performed for this study were otoscopy, tympanometry, DPOAEs and ABR. Results of this study indicated four subjects to have purely postsynaptic deafness with present DPOAEs and no measureable ABR waveforms. The other 26 cases showed obvious cochlear impairment documented by absent DPOAEs. The authors noted that based on the definition of auditory neuropathy (AN) being present OAEs and absent ABRs with poor speech discrimination, a diagnosis of AN is possible for the four patients with present OAEs and abnormal ABRs (Oysu et al., 2002).

The majority of research points to hyperbilirubinemia causing central auditory pathway lesion(s). However, with the introduction of AN, it has caused auditory researchers to speculate

on the loci of damage given the present OAEs and absent or abnormal ABR results in individuals with kernicterus. In the next section AN and hyperbilirubinemia is discussed further.

Auditory Neuropathy and Hyperbilirubinemia

The term auditory neuropathy (AN) was introduced in 1996 by Starr, Picton, Sininger, Hood, and Berlin. AN has been described as abnormal or absent ABR with normal outer hair cell function as tested by cochlear microphonic responses or OAE (Starr et al., 1996; Stein et al., 1996; Shapiro & Nakamura, 2001; Madden et al., 2002). Starr et al. (1996) indicated AN could be at the level of the inner hair cells, the synapse between inner hair cells and VIII nerve fibers, the ganglion neurons, the nerve fibers or any combination of the above. Based on the finding of normal otoacoustic emissions, the outer hair cells in the cochlea are presumed to be normal (Starr et al., 1996). Individuals with SNHL will typically have absent OAEs with absent ABR or elevated ABR threshold and prolonged latencies. However, in the presence of neural pathway damage or AN, outer hair cell function in the cochlea may be normal as indicated by present OAEs and an abnormal or absent ABR will indicate damage to the inner hair cells or the neural pathways beyond the inner hair cells (Shapiro & Nakamura, 2001).

AN was often observed in individuals with peak TSB values over 20 mg/dl (Shapiro & Nakamura, 2001; Shapiro, et al., 2002; Shapiro & Daymond, 2003). Shapiro & Nakamura (2001) reported frequent observation (33-50%) of hyperbilirubinemia in children diagnosed with AN. Case studies of children with hyperbilirubinemia who demonstrate audiological findings which support the definition of AN have been described in the literature (Stein et al., 1996; Deltenre, Mansbach, Bozet, Clercx, & Hecox, 1997; Simmons & Beauchaine, 2000). Similarly, studies of children diagnosed with AN and not having hyperbilirubinemia have also been

described (Buchman, Roush, Teagle, Brown, Zdanski, & Grose, 2006). Buchman et al. (2006) state AN is a generic diagnostic term that describes any condition in which gross discrepancy exists between measures of cochlear and neural function in the auditory system. Because AN appears to be used as a catch all term for normal OAEs and absent or abnormal ABR it leaves room for speculation and confusion.

Chisin et al. (1979) reported electrophysiologic study of children with deafness and children with hearing loss resulting from hyperbilirubinemia showed normal cochlear microphonic responses and abnormal or absent ABR. In a study by Kraus, Ozdamar, Stein, & Reed (1984), seven of 48 patients with no known brainstem damage were found to have no responses by ABR or absent waves III and V with pure tone sensitivity ranging from normal hearing to moderate impairment. Stein et al. (1996) evaluated four patients diagnosed with AN and found all four had elevated bilirubin levels warranting phototherapy treatment and one subject had an exchange transfusion.

Kraus et al. (1984) reported that several risk factors for hearing loss were present in the seven patients with paradoxical OAE and ABR findings including 50% had histories positive for perinatal asphyxia, 40% had hyperbilirubinemia, and 33% had head trauma. Shapiro & Nakamura (2001) surveyed parents of children diagnosed with classic kernicterus and found that ten of twelve children met clinical criteria for AN. Madden et al. (2002) evaluated 22 patients diagnosed with AN and found eleven of those patients had a history of hyperbilirubinemia plus several other risk factors for hearing loss including prematurity. Rance, Beer, Cone-Wesson, Shepherd, Dowell, King et al. (1999) evaluated 32 patients diagnosed with AN and found 16 patients to have a history positive for jaundice plus several other risk factors for hearing loss.

The combination of risk factors for hearing loss again makes it difficult to determine if hyperbilirubin is actually the cause of AN in these studies.

Amatuzzi, Northrop, Liberman, Thornton, Halpin, Herrmann et al. (2001) stated that the extent of damage to the hair cells and spiral ganglion neurons in neonatal hyperbilirubinemia remains controversial because of the dearth of pathologic material in infants without other potential risk factors for hearing loss; even where hyperbilirubinemia is not the suspect, the diagnosis of AN in infants is problematic. The term neuropathy means disease due to pathology in peripheral nerves and according to Rapin and Gravel (2006) in many cases the diagnosis of AN has been made without attempt to differentiate pathology in the VIIIth nerve from pathology in the other peripheral and central portions of the auditory pathway. As with many disorders, factors that cause hearing loss may be concomitant so there may be central damage from hyperbilirubinemia and peripheral damage from a different factor allowing AN to be diagnosed in the presence of hyperbilirubinemia. Hyperbilirubinemia is clearly a central disorder and AN is a peripheral disorder but the two can impact each other and they can co-exist (Musiek, 2010). However, hyperbilirubinemia is not a cause of AN unless there is retrograde degeneration of the cochlear nuclei to the auditory nerve (Rapin & Gravel, 2003; Musiek, 2010).

As indicated in this discussion, research has found a correlation between hyperbilirubinemia and prematurity indicating they are both significant risk factors for AN (Rance et al., 1999; Madden et al., 2002). Amin (2004) and Wennberg et al. (2006) stated that although the relationship between TSB and AN in the premature population is not firmly established, AN seems to be an important component of the bilirubin-induced brain-injury spectrum involving auditory pathways. AN must be considered when evaluating the auditory function of premature infants with hyperbilirubinemia.

Determining a Single Etiology of SNHL

It is difficult to determine if hyperbilirubinemia is the sole cause of SNHL when comorbidity of hearing loss risk factors often exist, more so in premature infants than full-term infants (Vohr, Widen, Cone-Wesson, Sininger, Gorga, Folsom et al., 2000). Amin (2004) indicated that because of the proximity of the auditory neural system to the cardio-respiratory center, ABR results may correlate with other brainstem functions such as control of heart rate and breathing. Correlations like this and others may skew research attempting to determine etiology of auditory dysfunction.

Hyperbilirubinemia has been proven to be a significant risk factor for auditory dysfunction in infants. Madden et al. (2002) evaluated the medical history of 22 patients diagnosed with auditory neuropathy and found a history of hyperbilirubinemia (50%), prematurity (45%), ototoxic drug exposure (41%), family history of hearing loss (36%), and neonatal ventilator dependence (36%). Billings and Kenna (1999) completed a retrospective chart review of 301 patients diagnosed with SNHL between the age of one month to 13 years of age. Birth factors including prematurity (8.5%), prolonged NICU stay (3.8%), and elevated bilirubin level (3.3%) were the most common etiologic factors for SNHL. De Vries, Lary, and Dubowitz (1985) retrospectively investigated premature infants <34 weeks of GA with peak TSB > 14 mg/dL and reported that hyperbilirubinemia carries approximately 30% risk of sensory hearing loss in high risk infants with birth weight < 1500 gm. Bergman, Hirsch, Fria, Shapiro, Holzman, and Painter (1985) evaluated 55 high-risk pre-term (≤36 weeks GA) infants and found hyperbilirubinemia (mean peak TSB 12 mg/dL) to be a significant predictor of sensory hearing loss along with number of days of respirator therapy and lowest serum sodium values.

The JCIH (2007) identified eleven risk indicators including those that are present during pregnancy and birth (congenital) and those that are acquired post-birth as a result of specific medical conditions or as a side affect of necessary medical interventions when treating an ill child. These risk indicators can be found in Table 1. Hyperbilirubinemia requiring exchange transfusion is one of those risk indicators. Additionally, the JCIH listed the following as risk factors to be considered when evaluating for neonatal hearing loss: chemotherapy and an illness or condition requiring admission of 5 days or greater to a NICU or any of the following regardless of length of stay: ECMO, assisted ventilation, exposure to ototoxic medications (gentimycin and tobramycin) or loop diuretics (furosemide/Lasix).

In several of the studies reviewed in this section, not all current risk factors for progressive or delayed onset of sensorineural hearing loss listed by the JCIH 2007 position statement were ruled out. This indicates that concomitant risk factors could be present which affect the auditory system. For instances, Sheykholeslami and Kaga (1999) only reported a review of the medical charts of the patients as neonates. It is unknown if risk factors for progressive or delayed onset hearing loss which may have been present between the neonate period and the age at which testing was completed (i.e., 4, 15, and 25 years of age) were evaluated.

CHAPTER 3

METHODS

The study was initiated following Human Subjects Committee (HSC) approval at University of Kansas Medical Center (KUMC). Refer to Appendix C for information regarding HSC approval. This prospective study was conducted by the Department of Hearing and Speech at KUMC in the KU Hospital NICU in Kansas City, Kansas under the direction of John A. Ferraro, Ph.D., the study's primary investigator. Assistance with subject recruitment was provided from Prabhu Parimi, M.D., the Neonatology Department Chair. The clinical investigator, Gabriel Bargen, completed all audiological testing, collected all data, and analyzed all data for the study.

Study Eligibility

To be study eligible, infants needed a GA of 28 to 34 weeks and/or normalized birth weight within the 95th percentile for infants 28 to 34 weeks GA (1559 to 3150 kgm). Normalized birth weight for the target GA range was included to account for the possibility of incorrectly estimated GA. All infants meeting these criteria that were made known to the clinical investigator were screened for study eligibility. To be considered for study eligibility, the infant had to be less than 72 hours old and be medically stable. Patients with elevated TSB levels and normal TSB levels were included in the study. The study group comprised premature infants in the target GA range with elevated TSB levels. The control group comprised premature infants in the same GA range who had normal levels of TSB. Study and control groups were determined post hoc to keep the clinical investigator blind to the bilirubin status.

Patients were excluded from the study if they were known to have: a family history of childhood hearing loss, a postnatal infection associated with SNHL (i.e., bacterial and viral meningitis), in utero infection such as cytomegalovirus, herpes, rubella, syphilis, and toxoplasmosis, a syndrome associated with hearing loss including Trisomy 21, CHARGE syndrome, atresia, Usher's syndrome, and a congenital defect associated with hearing loss including craniofacial anomalies (JCIH, 2007). Patients who had received or were receiving aminoglycoside therapy were included in the study provided that their trough levels were within the normal range of 0.6 – 2.0 (mcg/ml).

Once eligibility for the study was established the parent(s) was asked to sign an informed consent prior to testing. A Spanish interpreter was used to obtain informed parental consent from those subjects' parents whose primary language was Spanish.

Subjects

Subjects were enrolled in the study between December 2008 and August 2009. Ten preterm neonates, 5 male and 5 female, participated in this study. The subjects' mean GA was 31.5 weeks (SD = 2.07 weeks) with a range of 28 to 34 weeks. The mean birth weight was 1656.3 g (SD = 333.39 g) with a range of 1060 g to 2113 g. The racial demographic data indicated 7 Caucasian, 2 Hispanic, and 1 African American subjects. Table 2 summarizes the demographic characteristics of the subjects.

Auditory Brainstem Response Recordings

Equipment

A Biologic Systems Corporation NavigatorPRO (Natus Medical Incorporated, Mundelein, IL) unit, utilizing Auditory Evoked Potential program, version 6.2.0, was used to record and analyze the ABR.

Stimuli

A broadband click with 100 μsec electrical pulse that was alternating in polarity was utilized. The stimuli were presented at a rate of 33.3 clicks per second via EARtone-3A tubal insert earphones. Stimuli at each collection began at 80 dB nHL (using reference of 0 dB nHL as audiometric zero) and were reduced by approximately 20 dB for each subsequent recording until all ABR components disappeared into the noise floor. Once the components disappeared, the stimulus was increased by 5 dB until a repeatable ABR component, specifically wave V, was visible once again. Table 3 lists the ABR stimuli used for this study as well as the recording parameters described below.

Recording Parameters

Single-channel ABR was recorded with the use of three self-adhesive, disposable electrodes. Responses were recorded with the non-inverting (positive) electrode placed on the high forehead, inverting (negative) electrode placed on the ipsilateral-mastoid (test ear), and ground electrode placed on the contralateral-mastoid (non-test ear) or on the nape of the neck. The area of skin was prepared for electrode placement by exfoliating the skin using NuPrep Gel (D. O. Weaver and Company, Aurora, CO, USA) and a cotton swab to reduce skin impedance.

Impedance was measured prior to recording the ABR. Individual electrode impedance was required to be below 10,000 ohms and inter-eletrode impedance values were balanced to within 2000 ohms across electrodes.

This study used the collection parameters typically used by the KUMC Hearing and Speech Department Audiology Clinic. The EEG activity was band-pass filtered from 100 to 3000 Hz (6 dB/octave). A time window of 15 msec was used. Approximately 2000 stimulus presentations were averaged and the response was replicated at least twice at each level. Artifact rejection (±23.80 μV) was used to avoid contamination of the response by excessive myogenic activity. Additionally, testing was completed when infants were asleep to decrease myogenic artifact. Masking was not used during testing. As previously indicated, Table 3 lists the ABR recording parameters along with the ABR stimuli used for this study.

ABR Latencies

ABR absolute latencies and interwave intervals for waves I, III, and V were measured at 80 dB nHL using the cursor from the screen. Normative data have been determined for infant ABR peak latencies and interwave intervals (latency between two peaks) at various levels of stimulation (Cox, 1985; Gorga, Reiland, Beauchaine, Worthington, & Jesteadt; 1987). Data from this study were compared using conceptional age, which is the sum of GA and chronological age at the time of testing. The normative data from Gorga et al. (1987) were used to determine if latencies and interwave intervals were within normal limits. Normalized data could not be found for conceptional age less than 33 weeks. When the conceptional age was less than 33 weeks the normalized data from the 33-34 week group defined by Gorga et al. (1987)

were used. Latencies and interwave intervals for the ABR waveforms were verified by a blind observer experienced in the interpretation of evoked-potential responses.

Example response waveforms indicating the absolute latencies for the ABR are shown in Figures 1 and 2. The results in Figures 1 and 2 are for, subject 1, an infant born at 33 weeks GA with normal hearing. The results in Figures 1 and 2 were recorded during the second and third follow-up collections respectively. The stimulation level is indicated for each set of waveforms, beginning at 80 dB nHL. Waveforms where a response of wave I, III or V was judged to be present by the clinical investigator and the blind observer are indicated with coordinating wave I, III, or V markers.

ABR Thresholds

The procedures for finding ABR threshold in infants have been well documented (Elberling & Don, 1987; Ferraro, 1997; Sininger & Cone-Wesson, 2002). For this study the procedure for finding ABR threshold followed that defined by Ferraro in 1997. The averaging process began at 80 dB nHL, a level where all components of the response should be visualized. Two recordings were completed at this level to verify the response was repeatable. The stimulus level was then reduced by 20 dB for each subsequent recording until all ABR components disappeared into the noise floor. Once the ABR components disappeared, the stimulus was increased by 5 dB until a repeatable ABR component was visible once again. This component was wave V in all of the subjects. At least two recordings were completed at the last level where a repeatable wave V was visually observable and additionally at the next lowest level where it was not. The ABR threshold was estimated by wave V and defined as midway between the last repeatable and non-repeatable waveforms. Thresholds for the ABR tracings were verified by a

blind observer experienced in the interpretation of evoked-potential responses. There was 100% agreement between the clinical investigator and the blind observer.

Example response waveforms for the ABR are shown in Figures 1 and 2. As previously indicated, the results in Figures 1 and 2 are from subject 1, an infant born at 33 weeks GA with normal hearing. The results in Figures 1 and 2 were recorded during the second and third follow–up collections respectively. The ABR threshold was found to be 12.5 dB nHL for both Figures 1 and 2 as the last measurable wave V was found at 15 dB nHL and no replicable response was recorded at 10 dB nHL in both example responses.

Distortion Product Otoacoustic Emission Recordings

Equipment

A Biologic Systems Corporation NavigatorPRO (Natus Medical Incorporated, Mundelein, IL) unit, utilizing SCOUT OAE version 3.45.00, was used to record and analyze DPOAEs. DPOAE data were collected as DPgrams.

Stimulus Parameters

DPOAE data were collected in response to pairs of primary tones (f_1, f_2; $f_2 > f_1$) with f_2 stimulus presented at 2343 Hz (~2 kHz), 3046 Hz (~3 kHz), and 3749 Hz (~4 kHz) with an f_2/f_1 ratio of 1.2. The primaries L_1 and L_2 were presented at 65 dB SPL and 55 dB SPL respectively. For each f_2 stimulus, a minimum of 40 samples were collected. Measurement-based stopping rules were used during data collection. For each f_2 stimulus, averaging continued until a minimum DP level of -5 dB SPL and a minimum DP-noise floor (NF) level of 8 dB SPL was reached. If a NF of -17 dB SPL was reached averaging stopped. Additionally, averaging

stopped if a time out of 15 seconds, above 3 kHZ, or 20 seconds, at 3 kHz or below, had elapsed, whichever occurred first. Emission levels and noise floor levels at each f_2 stimulus were determined. A minimum DP-NF amplitude of 6 dB was required in order to use the recorded emission in the data analysis.

Example DP-grams are shown in Figures 3 and 4. The DP-grams in Figures 3 and 4 are recorded from the right ear of an infant born at 33 weeks GA, subject 1. The results for Figures 3 and 4 were recorded during baseline and the second follow-up collections respectively. Testing conditions when measuring the DP-grams from Figures 3 and 4 were identical except for the level of environmental noise. Figure 3 shows an increased noise floor when compared to Figure 4. In Figure 3, the DP response is greater than 6 dB above the noise floor only at 4000 Hz. In Figure 4, the DP response is greater than 6 dB above the noise floor at 2000, 3000, and 4000 Hz. As auditory function was found to be normal in this subject, the absent DPOAEs during baseline testing probably were not an indication of auditory dysfunction but more likely a result of poor testing environment.

DPOAE Procedure

A probe was placed into the outer ear canal and sealed snugly using a removable soft-rubber ear tip. The probe contained two speakers to produce the stimuli and a microphone to record the elicited emissions.

Bilirubin Monitoring

Bilirubin levels were measured per routine clinical practice by the KU Hospital NICU staff. Direct bilirubin levels and TSB levels were measured from blood samples. Bilirubin data

were collected by the clinical investigator from the electronic medical chart from each patient. TSB levels were documented until levels reached a plateau within the normal risk range.

Procedures

ABR and OAE evaluations were attempted four times on each subject and measurements were obtained from both ears if possible. However, due to the medical condition of the neonates (i.e., ventilation, head braces, etc.) at the time of data collection, testing could only be completed on one ear or not completed at all for several subjects. ABR and DPOAE data were collected while the infant was asleep.

Baseline testing was completed on approximately the 3rd postnatal day as TSB levels typically peak three to five days after birth. The first follow-up collection was attempted on approximately the 5th postnatal day. Third measurements were obtained once the bilirubin level had reached a plateau, indicating they had decreased to a level where bilirubin was no longer a risk, and phototherapy had been discontinued in those subjects who underwent phototherapy treatment. Finally, a fourth follow-up collection was completed two weeks after the third follow-up collection, to examine the sequelae of hyperbilirubinemia. Data regarding TSB levels were collected after all ABR and DPOAE tests were completed which kept the clinical investigator blind to subjects' bilirubin status.

Statistical Analysis

All analyses were performed with a type I error of 5%. Spearman's correlation coefficient (Johnson & Kuby, 2004) was used to investigate the first hypothesis for comparing the linear relationship of peak TSB levels and ABR absolute and interwave intervals latencies

globally. Spearman's correlation coefficient was used again to investigate the second hypothesis for comparing the linear relationship of peak TSB levels and ABR thresholds globally. Spearman's correlation coefficient was also used to investigate the third hypothesis for comparing the linear relationship of peak TSB levels and DPOAE responses globally.

CHAPTER 4

RESULTS

The general goal of this study was to determine the relationship between TSB levels and auditory function in pre-term infants. ABR absolute latencies, interwave intervals and thresholds were obtained along with DPOAE measurements to evaluate auditory function. These measurements were compared to the peak TSB levels of each subject.

Auditory Function Measurements

ABR and DPOAE measurements were attempted on each patient four separate times: baseline and three follow-up collections. Due to the medical condition of the subject, the level of environmental noise, or the elevated electrical artifact, testing of ABR and/or DPOAE could not be completed at all attempts. Refer to Appendix D for all raw ABR data listed in Tables D1, D2, and D3. Additionally, refer to Appendix D for all raw DPOAE data listed in Tables D4 and D5. ABR tracings from baseline and all follow-up collections are provided in Figures D1 through D4 in Appendix D. DP-grams from baseline and all follow-up collections are also provided in Figures D5 through D8 in Appendix D. The majority of the missing data occurred during the baseline and first follow-up collections. Based on the location of the missing data, analysis was completed using values collected from the second and third follow-up collections. Additionally, data from only the poorer ear was used in the analysis. The poorer ear was defined as that having the highest ABR threshold or, if the ABR threshold was equal between ears, the ear with

the most prolonged absolute latencies when the stimulus was presented at 80 dB HL. Using the poorer ear allowed for fewer missing data points.

ABR

To evaluate latency measurements ABRs were recorded at a stimulus level of 80 dB nHL. ABRs at this stimulus level were obtained from 9 of 10 ears in both the second and third follow-up collections. Table 4 lists the ABR absolute and interwave interval latencies from the worst ear recorded during the second and third follow-up collections. When subjects' individual latencies were compared to the normative data published by Gorga et al. (1987), 5 of 9 ears had prolonged absolute latency of wave V during the second follow-up. For the third follow-up collection, 2 of 9 ears had prolonged absolute latency of wave V. Additionally, during the second follow-up collection, 6 of 9 ears had prolonged III-V and I-V interwave intervals when compared to Gorga et al. (1987). For the third follow-up, 4 of 9 ears had prolonged III-V interwave intervals and 3 of 9 ears had prolonged I-V interwave intervals. When comparing the subjects' individual latencies the CA group that was closes to the infants CA was used. In several cases the CA of the individual was below that of the normative data CA group.

The average CA for the infants at the second follow-up collection was 33.5 weeks. The average CA for the infants at the third follow-up collection was 35.5 weeks. When the averages from the second follow-up collection were compared to the Gorga et al. (1987) normative data for infants in the 33-34 CA group no significant differences were found. Similarly, when the averages from the third follow-up collection were compared to the normative data for infants in the 35-36 CA group no significant differences were found. Table 5 lists means and standard deviations of wave I and V absolute latencies and the I-III, III-V, and I-V interwave interval

latencies for the study group and the corresponding normative data CA groups from Gorga et al. (1987).

DPOAE

DPOAE responses were obtained from 9 of 10 subjects at both the second and third follow-up collections. The DPOAE responses from the second and third follow-up that were used in the analysis coordinated with the ear that was determined to be the poorer ear according to the ABR recording. These DPOAE levels and NF levels recorded from the poorer ear are shown in Table 6. The recordings where the DP was found to be less than 6 dB above the noise floor are shown in bold print. Those recordings were not used in the data analysis as the emission was absent or the noise floor could have been obscuring the response.

TSB Monitoring

The medical chart of each subject was reviewed for bilirubin data after the collection of all audiological data. Peak TSB levels ranged from 4.9 to 10.9 mg/dl with a mean peak TSB of 7.91 mg/dL (standard deviation (SD) = 2.03 mg/dL). Among the subjects', total bilirubin levels peaked on 3, 4, 8, and 13 days of life with an average of 5.8 days. Peak TSB levels did not significantly correlate with either GA or infant birth weight. The correlations between peak TSB levels, GA and birth weight are listed in Table 2 along with the patient demographics previously described.

The phototherapy treatment thresholds of TSB (μmol/L) and B/A ratio (μmol/g) for groups based on birth weight developed by Hulzebos et al. (2008) were used in this study to assign subjects into the control group (standard risk) or the study group (increased risk). To

determine if subjects were in the control group or the study group, the total bilirubin to birth weight ratio was calculated using the peak TSB levels of each subject. These values are shown in Table 7.

According to the criteria established (i.e., that subjects with TSB level to weight ratio lower than 1mgm/100gm will be considered in the standard risk or control group), all 10 subjects were in the standard risk or control group. Figure 5 shows the scatter plot of the peak TSB levels to birth weight ratio for the subjects. The original intention of this study was to compare auditory function and peak TSB levels between the study group and the control group. However, as all subjects fell in the control group, auditory function and peak TSB levels were compared between all subjects.

Statistics

Spearman's correlation coefficient was used to investigate the first hypothesis which compared the linear association of peak TSB levels and ABR latencies. Nine patients had ABR absolute latencies and interwave interval latencies found to be present at 80 dB nHL during both the second and third follow-up collections. The poorer ear sample failed to prove a linear association between peak TSB levels and ABR absolute latencies and interwave interval for both the second and third follow-up collections. There was not a significant correlation between the peak TSB levels and any of the absolute or interwave interval latencies. Table 8 lists the means ±standard deviations along with the correlations and p-values calculated when comparing the linear association of peak TSB levels and ABR absolute and interwave interval latencies for the second and third follow-up collections. Figures 6a-b, 7a-b, 8a-b, and 9a-b show the scatter plots

of the peak TSB correlation with the latencies of wave III, V, I-V interwave interval, and III-V interwave interval respectively.

Spearman's correlation coefficient was also used to investigate the second hypothesis which compared the linear association of peak TSB levels and ABR thresholds. As with the previous investigation, thresholds were determined for 9 patients in both the second and third follow-up collections. The poorer ear sample failed to prove a linear association between peak TSB levels and ABR thresholds for both the second and third follow-up collections. There was not a significant correlation between the peak TSB levels and the ABR thresholds at either collection. Table 9 lists the means ±standard deviations along with the correlations and p-values calculated when comparing the linear association of peak TSB levels and ABR thresholds for this analysis. Figure 10a-b shows the scatter plot of the peak TSB and ABR thresholds for the second and third follow-up collections.

Spearman's correlation coefficient was also used to investigate the third hypothesis which compared the linear association of peak TSB levels and DPOAE responses globally. Frequencies used for calculations were 2000 Hz, 3000 Hz, and 4000 Hz. Those measurements in which the noise floor was at a level that would interfere with the distortion product emission (signal to noise ratio $\leq$ 6 dB) were not included in the calculations. Refer to Table 6 for the data used in the analysis. During the second follow-up collection, DPOAE responses were measured for 5 of 10 subjects at 2 kHz, 8 of10 subjects at 3 kHz, and 9 of 10 subjects at 4 kHz. During the third follow-up collection, DPOAE responses were measured for 9 of 10 subjects at all frequencies analyzed. The poorer ear sample failed to prove a linear association between peak TSB levels and DPOAE responses for both the second and third follow-up collections. There was not a significant correlation between the peak TSB levels and the DPOAE responses at

either measurement. Table 10 lists the means ±standard deviations along with the Spearman's correlations and p-values calculated when comparing the linear association of peak TSB levels and DPOAE responses for the second and third follow-up collections. Figure 11a-c shows the scatter plot of the peak TSB and DPOAE responses for the second follow-up collection. Figure 12a-c shows the scatter plot of the peak TSB and DPOAE responses for the third follow-up collection.

Case Report

Subject 6

When reviewing newborn hearing screening documents for the study subjects it was noted that all subjects passed the newborn hearing screening except subject 6. Subject 6 had normal auditory findings based on both ABR and OAE measurements during the study but failed the newborn hearing screening shortly following the study's final data collection. Because of this unusual occurrence the case report for this particular subject is described below.

Subject 6 was a female infant born at 30 weeks' GA. Birth weight was 1210 g which was below average for her age. She met all the eligibility requirements for the study, indicating she had no risk factors for congenital hearing loss or progressive or delayed on-set hearing loss except those being targeted by the study (i.e., prematurity, being in the NICU, hyperbilirubinemia). Serum bilirubin levels were 4.2 mg/dL on the second day of life, 4.9 mg/dL on the third day of life and peaking at 5.3 mg/dL on the fourth day of life. TSB levels of subject 6 were within the standard risk level and below the mean peak TSB for the group (7.91 mg/dL).

ABR measurements were obtained at baseline and the first follow-up session in the left ear and in both ears at the second and third follow-up collections. When compared to normative data from Gorga et al. (1987) the ABR absolute latencies and interwave intervals were essentially normal except for the second follow-up collection. At this session, wave V latency was prolonged along with III-V and I-V interwave intervals. However, the CA of subject 6 at the second follow-up session was 32 weeks and the CA of the normative data group was 33-34 weeks. This could have accounted for the prolongation of the latencies found during this session. Thresholds for all ABR measurements were found to be less than 25 dB nHL at all collections with the final ABR measurement thresholds of 7.5 dB nHL and 17.5 dB nHL in the left and right ear respectively.

DPOAE recordings were attempted in the left ear at baseline and the first follow-up collection but were not elicited. At the second follow-up collection, DPOAEs were measured at 4 kHz in the right ear but could not be elicited below 4 kHz in the right ear or at all in the left ear. At the third follow-up collection, DPOAEs were elicited in both ears at all frequencies evaluated at a signal to noise ratio greater than 6 dB. Auditory function was deemed to be within normal limits throughout the study for subject 6 when ABR and DPOAE data were reviewed.

The newborn hearing screening consisted of an ABR screen with the pass/refer criteria set at 30 dB nHL. The ABR screening responses for subject 6 indicated wave V was present at 60 dB nHL in both ears but absent at the 30 dB nHL screening level. The screening was completed 13 days after the final follow-up session for this study.

Subject 6 returned to the outpatient audiology clinic for a rescreening 20 days following the failed initial newborn hearing screen. Results reported the rescreen was passed. TEOAEs were elicited in both ears at a signal to noise ratio greater than 6 dB in at least 3 of 5 frequencies

screened. DPOAEs were also elicited in the left ear at a signal to noise ratio greater than 6 dB in 5 of 6 frequencies screened. DPOAEs were not attempted in the right ear. Results indicated subject 6 passed the ABR screen with wave V present at the 30 dB nHL screening level in both ears.

As described above, even though the subject 6 was found to have normal auditory function during the study, she failed the newborn hearing screening. One explanation for this finding is that the reliability of the study's auditory function evaluations was poor or the reliability of the auditory screening was poor. However, a more likely reason is that the subject may have had temporary auditory dysfunction at the time of the newborn hearing screening that dissipated prior to the newborn hearing rescreen.

CHAPTER 5

DISCUSSION

The current study had one main goal: to determine the relationship between peak TSB levels and auditory function in preterm neonates. Three hypotheses were tested to meet this goal: 1) as peak TSB levels worsen the ABR will indicate an increase in absolute latency of wave III and/or wave V and interwave latency of III-V and/or I-V in premature neonates, 2) as peak TSB levels improve the ABR threshold will improve in premature neonates, and 3) DPOAE responses will be unaffected by the improvement or deterioration of peak TSB levels in premature neonates.

Results from the Present Study

The study used the currently accepted peak TSB level to birth weight ratio to determine which subjects' bilirubin levels were within the range of standard risk as opposed to those determine to be at an increased risk. The cutoff placed those subjects with ratios lower than 1mgm/100gm in the control group, which for this study included all the subjects. All subjects were found to have normal auditory function as well. This demonstrated that the ratio cutoff of 1mgm/100gm was appropriate as the auditory function of all subjects below this level was not negatively affected by bilirubin as expected.

The first hypothesis of this study was that as peak TSB levels worsened the ABR indicated an increase in absolute latency of wave III and/or wave V and interwave latency of III-V and/or I-V in premature neonates. Results pertaining to this hypothesis are shown in Table 8 and Figures 6a-b, 7a-b, 8a-b, and 9a-b. This correlation did not reach statistical significance

suggesting that there was no evidence to support the hypothesis that ABR latencies would be prolonged as peak TSB levels worsen. When the average latencies of wave V and the interwave intervals were compared to the normative data from Gorga et al. (1987) as a group, all data fell within one standard deviation of the mean. This finding indicated that the ABR absolute latency and interwave interval measurements as a group were representative of normative responses.

Examination of the individual data revealed a prolonged wave V in 5 of the 9 ears analyzed from the second follow-up collection and in 2 of the 9 ears analyzed from the third follow-up collection. Additionally, the III-V and I-V interwave intervals were prolonged in 6 of the 9 ears analyzed from the second follow-up collection. From the third follow-up, 4 of 9 ears had prolonged III-V interwave intervals and 3 of 9 had prolonged I-V interwave intervals. Three of the ten subjects were below the conceptional age of the normative data during the second collection and one was still below during the third collection. These subjects account for the majority of the prolonged data. The latencies from these subjects may not have been considered prolonged if they could have been compared to normative data from a more similar conceptional age group; however, these data are not available.

The second hypothesis of the study was that as peak TSB levels improved/worsened in premature neonates the ABR threshold followed suit. Results pertaining to this hypothesis are shown in Table 9 and Figures 10a-b. This correlation did not reach statistical significance suggesting that there was no evidence to support the hypothesis that ABR thresholds would worsen as peak TSB levels worsen.

Examination of the individual data revealed all ABR thresholds from the baseline data collection and the three follow-up collections were below 30 dB nHL except one. Subject 9 had a threshold of 37.5 dB nHL at baseline collection. This subject did have present DPOAEs at 3

kHz and 4 kHz at baseline collection in the same ear. Subject 9 had a peak TSB level of 6.9 mg/dL which was below the group average of 7.91 mg/dL. The TSB level for subject 9 peaked on day 4 of life; one day after baseline data was collected. The first follow-up collection took place 3 days after baseline collection. ABR threshold was found to be 17.5 dB nHL at the first follow-up collection in the same ear for subject 9. Subsequent follow-up ABR testing in that ear continued to estimate threshold to be below 30 dB nHL. Whatever had caused the threshold to worsen during baseline measurements did not appear to effect follow-up measurements.

The third hypothesis of the study was that DPOAE responses were unaffected by the improvement or deterioration of peak TSB levels in premature neonates. Results pertaining to this hypothesis are shown in Table 10 and Figures 11a-c and 12a-c. This correlation did not reach statistical significance suggesting that there was no evidence to support the hypothesis that DPOAE responses were unaffected by peak TSB level changes.

Comparison to Previously Published Literature

Research has shown that hyperbilirubinemia in pre-term infants is more prevalent, more severe, and its course more protracted than in term neonates (Billings, Cole, & Lathe, 1954; Harris, 1961; Watchko, 2000). The threshold at which bilirubin begins to affect auditory function in preterm neonates is unclear. Unfortunately, due to the limited study sample and lack of enrollment of infants with elevated bilirubin levels this study failed to shed light on this issue.

Research has indicated that preterm infants are affected by lower peak TSB levels than when compared to full term infants. A study by van de Bor, van Zeben-van der Aa, Verloove-Vanhorick, Brand, and Ruys (1989) investigated the relationship between maximal TSB concentration and neurodevelopmental outcome. The mean peak TSB level for children with

normal neurodevelopment was 10.3 mg/dL while the mean peak TSB level for those with a minor handicap was 10.7 mg/dL and for those with major handicap was 11.4 mg/dL (van de Bor et al., 1989). The results of the present study had a mean peak TSB of 7.91 mg/dL with a range of 4.9 to 10.9 mg/dL. Two subjects had peak TSB levels above the 10.3 mg/dL threshold for children with normal neurodevelopment reported by van de Bor et al. (1989). Future studies may be warranted that evaluate neurodevelopment in these preterm infants with elevated peak TSB levels.

The peak TSB to birth weight ratio used to determine whether study participates were in the control group or study group is one of the tools used to determine if infants need phototherapy in the KUMC NICU (Parimi, 2009). When the peak TSB to birth weight ratios were determined for the present study all subjects fell below the 1.0 cut off which placed all subjects in the control group. However, all babies in the study received some amount of phototherapy. Other determinates aside from peak TSB to birth weight ratio appear to be used for the initiation of phototherapy in the KUMC NICU. Using lower TSB thresholds for hyperbilirubin intervention in infants in NICUs is common (Bhutani and Johnson, 2004). With the increasing survival of infants born prematurely, Cashore (2000) stated it is difficult to specify what concentrations of TSB are too high. Therefore no single recommendation may serve the needs of all nurseries and their premature patients. The KUMC NICU appears to be using phototherapy to treat increased bilirubin liberally but properly when compared to management of bilirubin by other NICUs described by the literature.

Study Strengths

One of the strengths of this study included a standardized assessment method to ensure reliable evaluations across all subjects and for all evaluations on each subject. The same clinical investigator, Gabriel Bargen, completed all the evaluations on every subject. The clinical investigator made all attempts to use the same protocol for each evaluation of auditory function.

A second strength of this study included the enrollment rate. Study enrollment occurred between January 2009 and August 2009. During this time 62 infants with GA of 28 to 34 weeks were admitted to the KU Hospital NICU. The medical status and knowledge of concomitant risk factors for hearing loss for all 62 infants were unknown. Therefore, all 62 infants may not have been eligible for the study. However, based solely on the GA criteria, 10 of 62 infants eligible for the study were enrolled. This gave the study a 16% enrollment rate.

Grundmeier, Swietlik, and Bell (2007) evaluated subject enrollment when patients were informed of studies by their clinician using electronic health records. They found that eleven studies with a potential subjects ranging from 17 to 1,162 only had a small portion of the subjects actually enroll in the studies. The enrollment rate ranged from 3% to 25% with a median of 11%. Mosis, Deileman, Stricker, van der Lei, and Sturkenboom (2006) assessed patient recruitment when physicians were informed of eligible subjects in their database automatically. They found that of 170 eligible study subjects only 20 were enrolled yielding an 11.8% enrollment rate. In 1999, CenterWatch, a source for clinical trials information, reported that 2.8 million individuals completed initial screening for industry-sponsored clinical trials. An estimated 21% of those who responded to these recruitment promotions showed up for initial screening, and only 7% were enrolled in studies (Kroll, 2001). When compared to these studies, the 16% enrollment rate of the current study would be considered high.

The high follow-up rate of 97.5% was also a strength of this study. Baseline and two follow-up collections of auditory function were attempted on all subjects. One subject, subject 3, was discharged before the final follow-up measurements could be completed.

An additional strength of this study included blinding of the clinical investigator to increase study validity. The TSB levels of each subject were unknown to the clinical investigator until the completion of all auditory function evaluations. This decreased potential bias that could have occurred if the clinical investigator was aware of the subjects' bilirubin status during the measurement of auditory function.

Study Limitations

There were several limitations to this study that need to be taken into account when interpreting the data. First of all, the number of subjects was limited. Although the enrollment rate was relatively good (16%) when compared with other clinical studies, the target enrollment of 45 subjects was not met. This factor could have been partially due to reduced admissions of patients eligible for the study based on GA range. Active enrollment began in January. Two subjects were enrolled in February, one subject was enrolled in April, two patients were enrolled in both June and July, and three subjects were enrolled in August. Additionally, the communication between the clinical investigator enrolling subjects and the doctors and nurses informing the clinical investigator when eligible subjects were admitted to the NICU was limited. A potential reason for this was the clinical investigator was only on campus three days a week to physically check NICU admissions. The other four days of the week the clinical investigator relied on the attending physician (co-primary investigator), to make known eligible admissions. Given the physician's demanding schedule, many eligible patients were not made

known to the primary investigator until they were older than 72 hours of life. Contact was made with the nurses in the NICU and a presentation was provided indicating the limited enrollment timeline; however, no eligible admissions were made known by the unit nurses. Because of the limited amount of time to enroll patients (i.e., prior to 72 hours of life) this line of communication needed to be open with contact being made daily or more by the investigating clinician and both the physicians and the nurses involved with KUMC NICU admissions.

Incomplete data for both ears at baseline and follow-up sessions also was a limitation to this study. The medical condition of the subjects contributed to this condition. Several babies required assistance breathing via a ventilator and therefore testing could only be completed on one ear as the infant could not be turned. Most babies were receiving phototherapy treatment during one or more of the auditory function evaluations. Phototherapy treatment produces cyclical energy which interferes with ABR testing. The location of the babies was also a contributing factor. All babies were in incubators or radiant warmers for baseline and several follow-up evaluations. Incubators and radiant warmers contain a high level of cyclical energy (i.e., overhead electric heating elements, warming blanket, warm moistened air) which as previously mentioned interferes with ABR testing.

Another limitation is the environment in which auditory tests were completed. The NICU environment along with incubators and respiratory support equipment in the NICU combine to create a high level of environmental noise which can interfere with OAE and ABR testing. It has been well documented that the acoustic environment in the average NICU can often fluctuate between 40 and 90 dB(A) with impulses as high as 140 dB(A) (Darcy, Hancock, & Ware, 2008; Goldson, 1999; Kreuger, Wall, Parker, & Nealis, 2005; Walsh, McCullough, & White, 2006). Additionally, the sound level of the incubator as well as the method of respiratory

support can raise the level of noise in the testing environment for preterm infants. Lasky & Williams (2009) reported the noise levels of incubators and respiratory support exceeded noise recommendations of the American Academy of Pediatrics (AAP). They found that only 5.51% of the time were the sound levels within the recommendations made by the AAP in 1997 (i.e., noise levels <45 dB(A). If the sound levels in the NICU environment outside and within the incubators is elevated it will in turn increase the level of the noise floor during testing. Having an increased noise floor can interfere with both OAE and ABR testing.

Future Studies

Further research into the pre-term population regarding the effect of increased bilirubin on auditory function is warranted. This study provided no evidence of significant correlation between ABR measurements and peak TSB levels or DPOAE measurements and peak TSB levels in preterm infants with normal auditory function and peak TSB levels within the range of standard risk. Continuing the study with preterm infants that have confirmed elevated TSB levels would be needed to determine if a trend between the ABR measurements or DPOAE responses and TSB levels is present. Clearly, further research is needed to determine the relationship between bilirubin levels and auditory function in preterm infants.

CHAPTER 6

CONCLUSION

The present investigation was undertaken because the available evidence regarding the level at which bilirubin affects the auditory system in premature neonates is lacking. The present study addressed this need by evaluating the relationship between TSB levels and auditory function in pre-term infants. Auditory function was evaluated using ABR and DPOAE measurements. Ten infants born with a GA between 28 to 34 weeks were included in this study. All ten subjects had bilirubin levels falling in a standard risk group according to peak TSB levels to birth weight ratios. The ten subjects were found to have normal auditory function. In this study there was no evidence to support the hypothesis that as peak TSB levels worsen the ABR will indicate an increase in absolute latency of wave III and/or wave V and interwave latency of III-V and/or I-V in premature neonates. Additionally, there was no evidence to support the hypothesis that as peak TSB levels improve the ABR threshold will improve in premature neonates. Finally, in this study there was no evidence to support the hypothesis that DPOAE responses will be unaffected by the improvement or deterioration of peak TSB levels in premature neonates. The study did provide baseline data that can be used in future research evaluating auditory function in preterm infants with confirmed hyperbilirubinemia. No assumptions can be made regarding criteria for initiating intervention aimed at ameliorating the effects of high bilirubin levels on auditory function in preterm infants from the results of this study. Further research including subjects with increased bilirubin is needed to determine if and when auditory function of preterm infants is affected by elevated bilirubin.

APPENDIX A

TABLES

Table 1: The Joint Committee on Infant Hearing 2007 position statement's risk factors for infant hearing loss.

1. Parental or caregiver concern regarding hearing, speech, language, and/or developmental delay.
2. Family history of permanent childhood hearing loss.
3. Stigmata or other findings associated with a syndrome know to include a sensorineural or conductive hearing loss or eustachian tube dysfunction (i.e., Trisomy 21; Pierre Robin syndrome; CHARGE syndrome; atresia; Rubinstein-Taybi syndrome; Stickler syndrome; Goldenhar syndrome).
4. Craniofacial anomalies, including those that involve the pinna, ear canal, ear tags, ear pits, and temporal bone anomalies.
5. Postnatal infections associated with SNHL (i.e., bacterial and viral meningitis).
6. In utero infection such as cytomegalovirus, herpes, rubella, syphilis, and toxoplasmosis.
7. Neonatal indicators (i.e., hyperbilirubinemia at a serum level requiring exchange transfusion; persistent pulmonary hypertension associated with mechanical ventilation; use of extracorporeal membrane oxygenation).
8. Syndromes associated with progressive hearing loss (i.e., neurofibromatosis; osteopetrosis; Usher's syndrome).
9. Neurodegenerative disorders (i.e., Hunter syndrome; Friedreich's ataxia; Charcot-Marie-Tooth syndrome).
10. Head trauma
11. Recurrent or persistent otitis media externus for at least 3 months.

Table 2: Subject demographics (mean ± standard deviation) and Spearman's correlation between peak TSB level and birth weight and GA.

	Mean ± SD	Correlation to peak TSB	p-value
Subjects (Male/Female)	n=10 (5/5)		
Birth weight (grams)	1656.3 ± 333.40	0.322	0.364
Gestation (weeks)	31.5 ± 2.07	0.257	0.474
Peak total serum bilirubin (mg/dL)	7.91 ± 2.03		

Table 3: Auditory brainstem response collection parameters used for this study.

Recording parameters	
Channel	1
# Averages	2000
Timebase	15 msec
Filter (bandpass)	0.1–3.0 kHz, 6 dB/octave
Artifact reject	±23.80 µV

Stimuli	
Type	Broadband click (100 µs electrical pulse)
Transducer	Tubal insert (EARtone – 3A)
Polarity	Alternating
Level	Begin at 80 dB nHL then decrease level by 20 dB until no discernable wave V identifiable, then up 5-10 dB to estimate threshold
Rate	33.3/second
Masking	none

Table 4: ABR absolute latencies and interwave interval latencies from the worst ear at the second and third follow-up collections. Mean and standard deviation for each absolute latency and interwave interval is also provided. Stimulus level was presented at 80 dB nHL.

	2nd Follow-up						3rd Follow-up					
Latency	I	III	V	I-III	III-V	I-V	I	III	V	I-III	III-V	I-V
Subject												
1	1.95	5.01	7.82	3.12	3.00	6.13	1.76	4.64	7.51	2.87	2.88	5.75
2							1.70	4.14	6.82	2.44	2.69	5.13
3	1.95	4.51	6.95	2.56	2.44	5.00						
4	2.20	4.64	6.82	2.44	2.19	4.63	1.64	4.39	6.64	2.75	2.25	5.00
5	1.76	4.89	7.82	3.12	2.94	6.06	1.89	4.64	7.26	2.75	2.63	5.38
6	1.64	4.45	7.64	2.81	3.19	6.00	1.95	4.32	7.20	2.37	2.88	5.25
7	1.89	4.76	7.82	2.87	3.06	5.93	1.51	4.14	7.20	2.62	3.06	5.69
8	1.58	4.20	7.39	2.62	3.19	5.81	1.85	4.51	6.95	2.66	2.44	5.10
9	1.76	4.70	7.82	2.94	3.13	6.06	1.64	4.76	7.32	3.12	2.56	5.69
10	1.89	4.39	6.95	2.50	2.56	5.06	2.85	5.39	7.57	2.54	2.19	4.72
Mean	1.85	4.62	7.45	2.78	2.86	5.63	1.87	4.55	7.16	2.68	2.62	5.30
SD	±0.19	±0.26	±0.43	±0.26	±0.37	±0.57	±0.39	±0.38	±0.31	±0.23	±0.29	±0.36

Table 5: The means and standard deviations (mean ± SD) for the latencies of various waves and interwave intervals for the second and third follow-up collections compared to the normative data from Gorga et al. (1987) for the corresponding average CA. Stimulus level is 80 dB nHL for all latencies and latencies are given in msec.

	Gorga et al. (1987)	2nd Follow-up	Gorga et al. (1987)	3rd Follow-up
	CA=33-34	CA = 33.5	CA=35-36	CA = 35.5
	n=38	n=9	n=144-150	n=9
Absolute Latency				
I	1.779	1.85	1.781	1.87
	±0.304	±0.19	±0.261	±0.39
V	7.054	7.45	7.019	7.16
	±0.394	±0.43	±0.375	±0.31
Interwave Intervals				
I-III	2.863	2.78	2.848	2.68
	±0.283	±0.26	±0.269	±0.23
III-V	2.411	2.86	2.39	2.62
	±0.259	±0.37	±0.25	±0.29
I-V	5.274	5.63	5.24	5.30
	±0.356	±0.57	±0.357	±0.36

Table 6: DPOAE levels (dB SPL) and noise floor levels (dB SPL) for subjects' worst ear at the second and third follow-up collections for 2000, 3000, and 4000 Hz. Subjects GA (weeks) and peak TSB (mg/dL) are provided for reference.

			2nd Follow-up						3rd Follow-up					
		Peak	2kHz		3kHz		4kHz		2kHz		3kHz		4kHz	
Subject	GA	TSB	DP	NF	DP	NF	DP	NF	DP	NF	DP	NF	DP	NF
1	33	10.9	2.3	-6.2	6.3	-8.7	2.4	-8.1	11.5	2.0	10.9	1.4	12.3	-3.8
2	33	9.0	12.9	2.0	10.8	-4.3	10.9	-6.1	15.4	1.4	18.6	3.1	16.2	0.3
3	34	8.9	13.0	-7.8	9.7	-11.4	12.3	-17.2						
4	34	4.9	**-3.9**	**-7.6**	-1.3	-11.1	-1.9	-10.5	4.1	-2.8	8.1	-1.3	8.0	-1.6
5	28	8.6	**7.3**	**10.5**	8.6	-1.0	3.6	-6.5	24.8	16.2	9.0	-4.5	7.0	-1.6
6	30	5.3					**2.5**	**0.8**	8.7	1.3	1.9	-6.9	11.4	2.1
7	29	6.7	**6.3**	**3.1**	**-0.5**	**-6.0**	10.1	2.0	12.7	4.1	12.3	2.1	15.4	4.8
8	32	10.5	9.6	-2.7	10.9	-5.9	14.4	-9.9	13.0	-0.9	18.0	-9.9	16.6	-7.3
9	31	6.9	11.6	-5.7	15.9	-7.2	11.0	-6.8	14.2	0.6	12.9	2.7	4.5	-9.6
10	31	7.4	**7.9**	**8.6**	5.1	-3.6	5.5	-5.0	7.0	-2.0	12.6	4.4	11.3	-2.0

*__Bold__ = SNR < 6 dB SPL (measurement not used in analysis)

Table 7: Peak total bilirubin (mg/dL) to birth weight (g) ratios.

Subject	Birth Weight	Peak TSB	bw/100	TB/(bw/100)
1	1830	10.9	18.3	0.596
2	1610	9.0	16.1	0.559
3	1930	8.9	19.3	0.461
4	1930	4.9	19.3	0.254
5	1060	8.6	10.6	0.811
6	1210	5.3	12.1	0.438
7	1600	6.7	16.0	0.419
8	2113	10.5	21.13	0.497
9	1800	6.9	18	0.383
10	1480	7.4	14.8	0.500

Table 8: Means ± standard deviations, Spearman's correlations, and p-values for comparison between ABR absolute latencies and interwave intervals and peak TSB levels for the second and third follow-up collections.

	2nd Follow up			3rd Follow up		
	mean±SD	Correlation	p-value	mean±SD	Correlation	p-value
Latency						
I	1.85 ±0.19	-0.084	0.829	1.87 ±0.39	0.243	0.529
III	4.62 ±0.26	0.100	0.798	4.55 ±0.38	0.244	0.527
V	7.45 ±0.43	0.227	0.556	7.16 ±0.31	0.293	0.444
I_III	2.78 ±0.26	0.377	0.318	2.68 ±0.23	0.192	0.620
III_V	2.86 ±0.37	0.084	0.831	2.62 ±0.29	0.059	0.881
I_V	5.63 ±0.57	0.335	0.379	5.30 ±0.36	0.226	0.559

Table 9: Means ± standard deviations, Spearman's correlations, and p-values for comparison between peak TSB levels and ABR thresholds (dB nHL) for the second and third follow-up collections.

	2nd Follow up			3rd Follow up		
	mean±SD	Correlation	p-value	mean±SD	Correlation	p-value
Threshold	18.05 ±5.27	-0.208	0.591	19.44 ±4.64	-0.201	0.604

Table 10: Means ± standard deviations, Spearman's correlations, and p-values for comparison between peak TSB levels and DPOAE responses for the second and third follow-up collections.

	2nd Follow up			3rd Follow up		
	mean±SD	Correlation	p-value	mean±SD	Correlation	p-value
Frequency						
2 kHz	9.88 ±4.45	-0.700	0.188	12.38 ±5.91	0.483	0.187
3 kHz	8.25 ±5.06	0.262	0.531	11.59 ±5.08	0.550	0.125
4 kHz	7.59 ±5.42	0.300	0.433	11.41 ±4.26	0.450	0.224

APPENDIX B

FIGURES

Figure 1: Sample ABR waveforms from subject 1, a normal hearing infant, in response to a click stimulus recorded during the second follow-up collection. The infant had a peak TSB level of 10.9 mg/dL. The stimulation level is indicated to the left of each waveform group: 80 dB nHL, 60 dB nHL, 40 dB nHL, 20 dB nHL, 15 dB nHL, and 10 dB nHL. Waveforms where a response of wave I, III or V was judged to be present by the clinical investigator and the blind observer are indicated with coordinating wave I, III, or V markers.

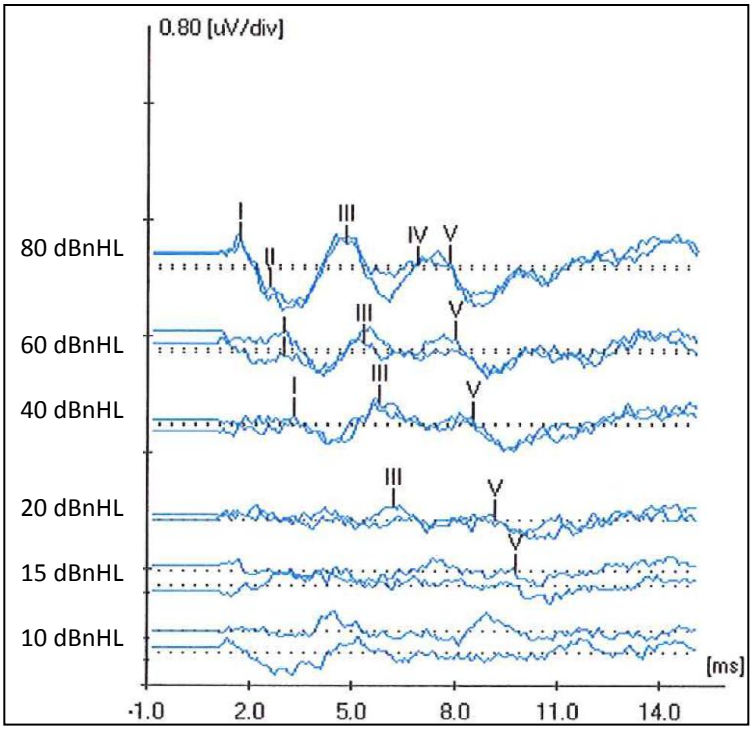


Figure 2: Sample ABR waveforms from subject 1, a normal hearing infant, in response to a click stimulus recorded during the third follow-up collection. The infant had a peak TSB level of 10.9 mg/dL. The stimulation level is indicated to the left of each waveform group: 80 dB nHL, 60 dB nHL, 40 dB nHL, 20 dB nHL, 15 dB nHL, and 10 dB nHL. Waveforms where a response of wave I, III, or V was judged to be present by the clinical investigator and the blind observer are indicated with coordinating wave I, III, or V markers.

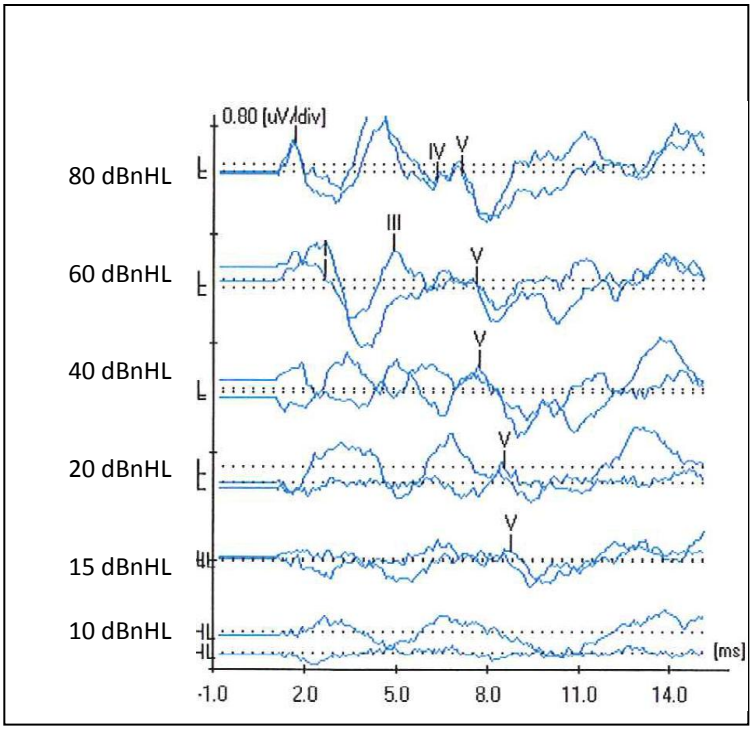


Figure 3: Sample DP-gram from the right ear of an infant born at 33 weeks GA, subject 1. The DP-gram was recorded at baseline testing. The infant had a peak TSB level of 10.9 mg/dL. Robust emissions with the DP more than 6 dB above the noise floor were detected at only 4000 Hz. An ABR recorded at the same time found the infant to have normal hearing. As described in the document, since auditory function was found to be normal in this subject, the absent DPOAEs found at this testing probably were not an indication of auditory dysfunction but more likely an indication of poor testing environment.

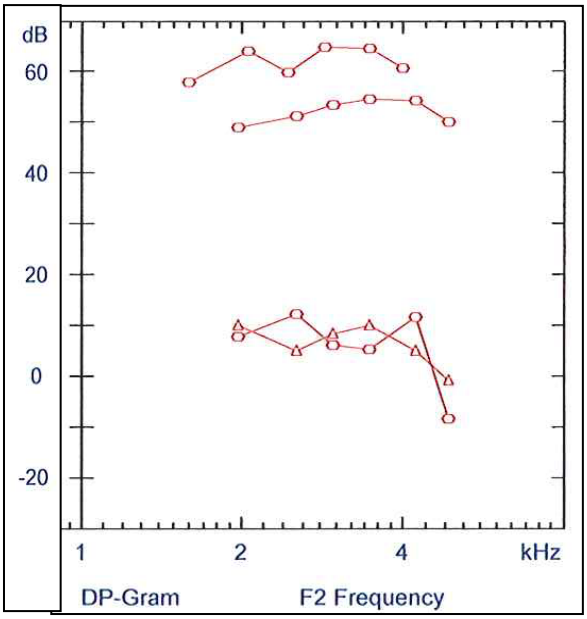


Figure 4: Sample DP-gram from the right ear of an infant born at 33 weeks GA, subject 1. The DP-gram was recorded at the second follow-up collection. The infant had a peak TSB level of 10.9 mg/dL. Robust emissions with the DP more than 6 dB above the noise floor were detected at 2000, 3000, and 4000 Hz. An ABR recorded at the same time found the infant to have normal hearing.

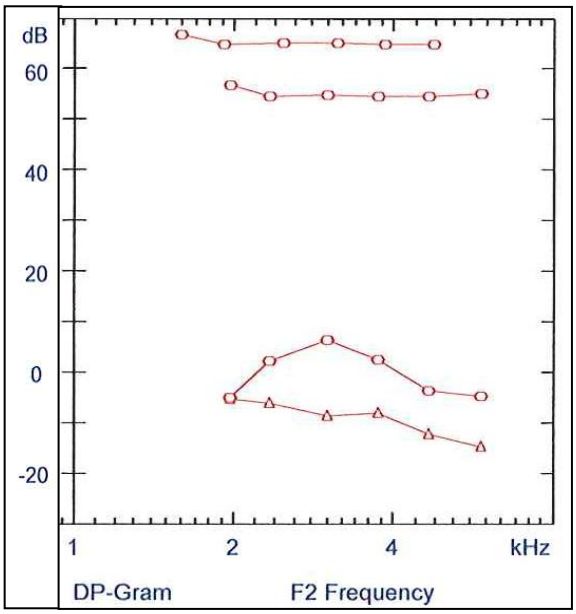


Figure 5: Scatter plot of peak TSB levels to birth weight ratio for all subjects. The 1 mgm/100gm threshold for group determination is highlighted.

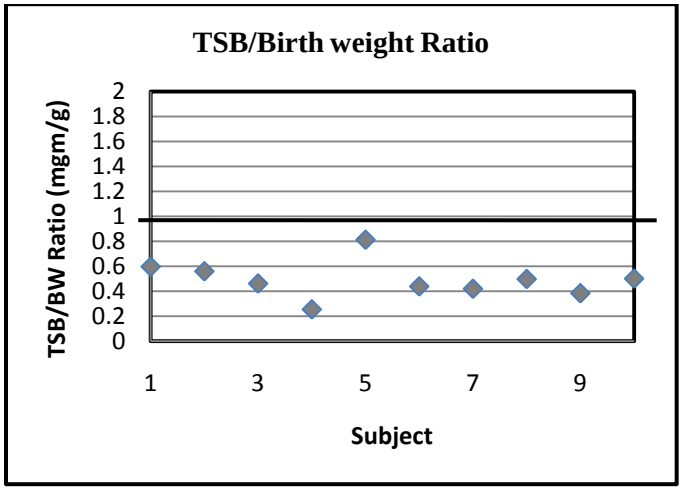
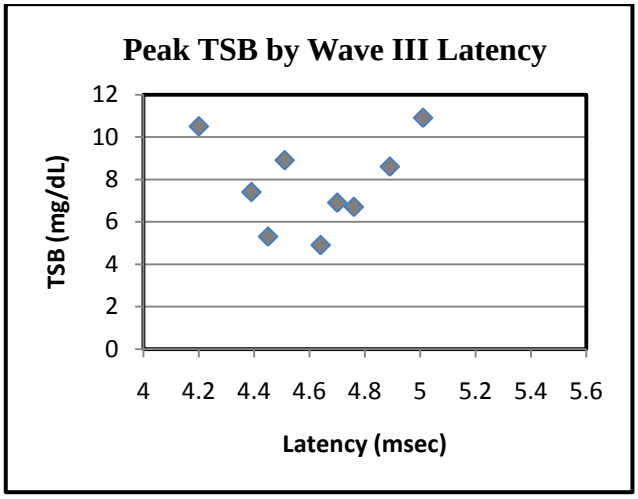


Figure 6 a-b: Scatter plots of peak TSB levels and ABR wave III latencies from the (a) second and (b) third follow-up collections.

(a)



(b)

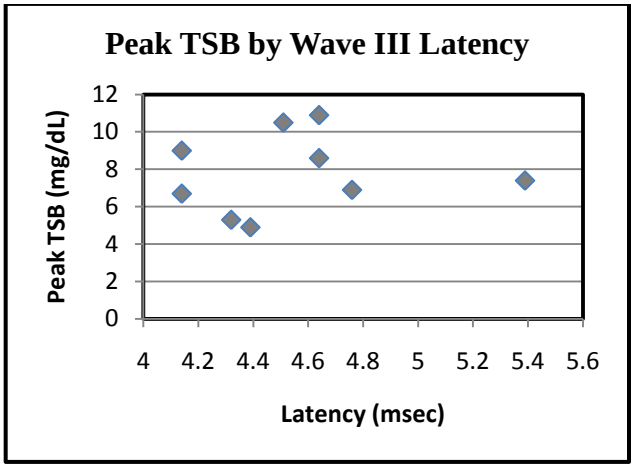
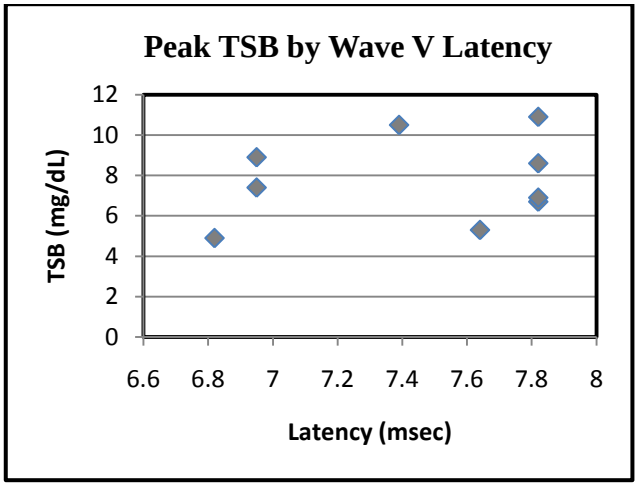


Figure 7 a-b: Scatter plots of peak TSB levels and ABR wave V latencies from the (a) second and (b) third follow-up collections.

(a)



(b)

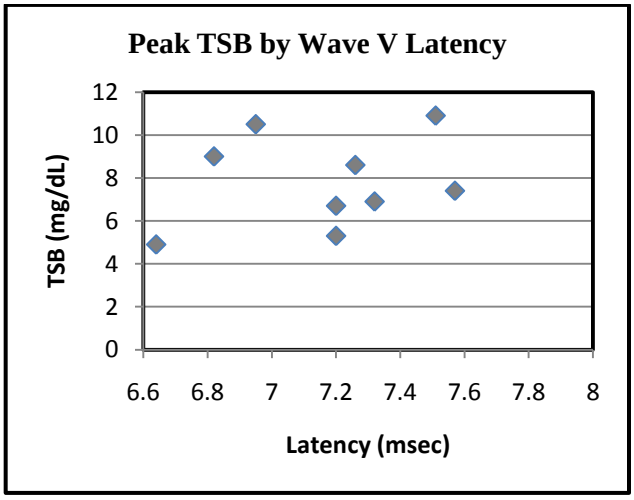
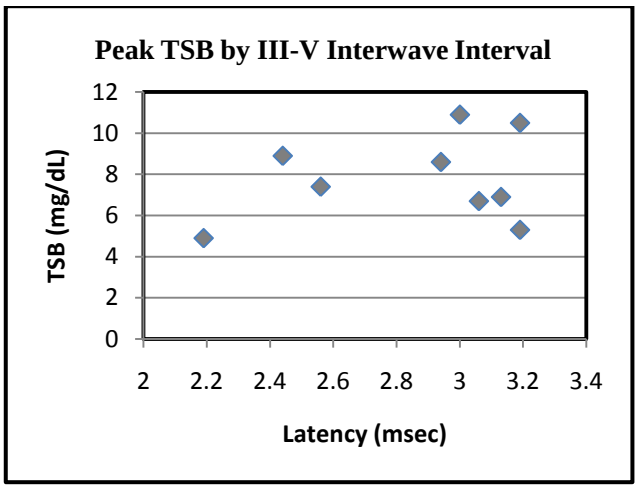


Figure 8 a-b: Scatter plots of peak TSB levels and ABR interwave interval III-V latencies from the (a) second and (b) third follow-up collections.

(a)



(b)

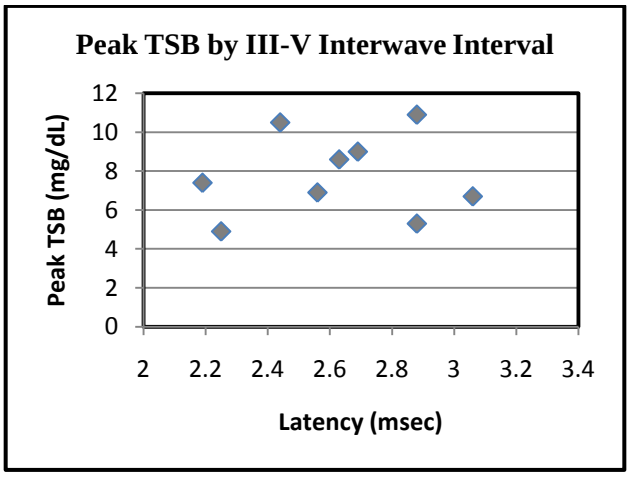
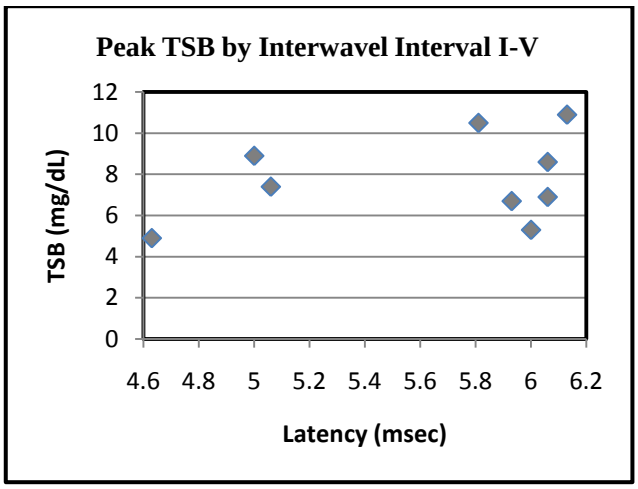


Figure 9 a-b: Scatter plots of peak TSB levels and ABR interwave interval I-V latencies from the (a) second and (b) third follow-up collections.

(a)



(b)

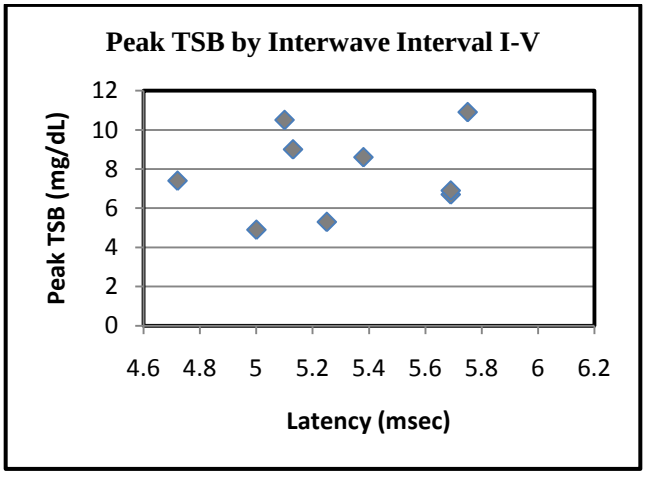
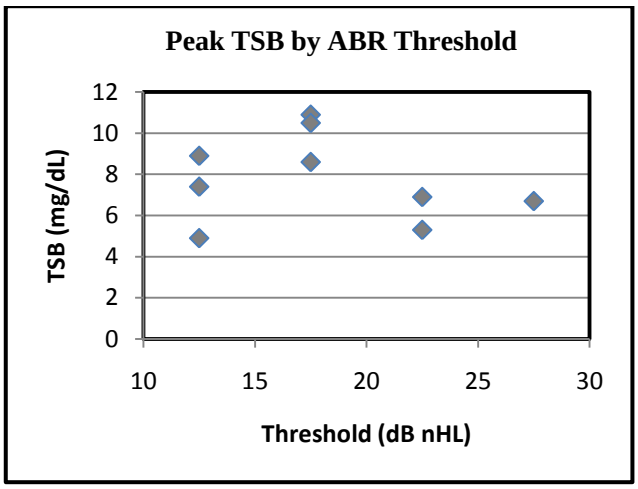


Figure 10 a-b: Scatter plots of peak TSB levels and ABR thresholds from the (a) second and (b) third follow-up collections.

(a)



(b)

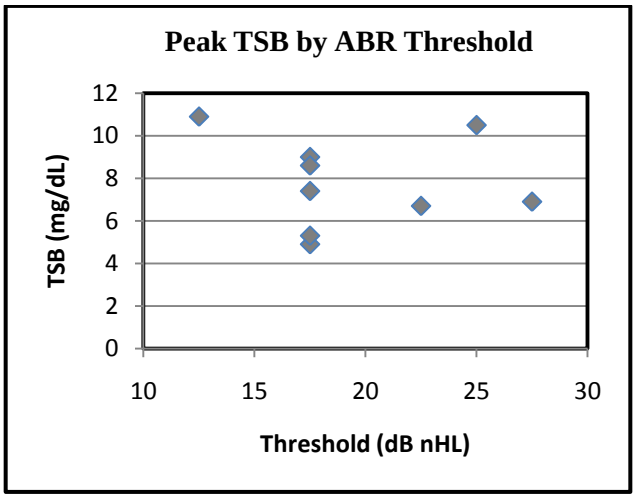
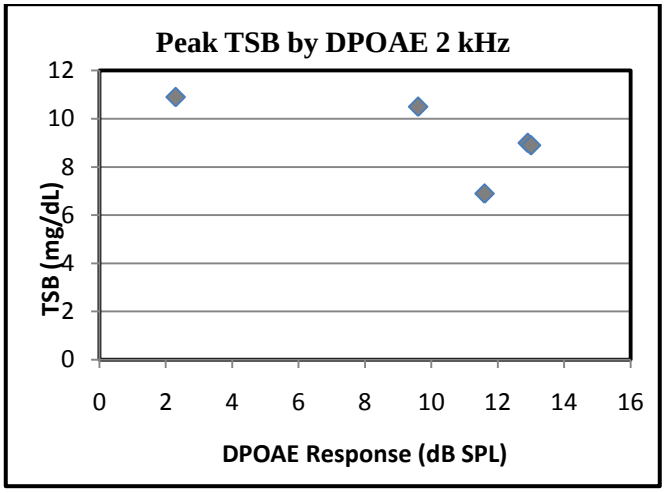
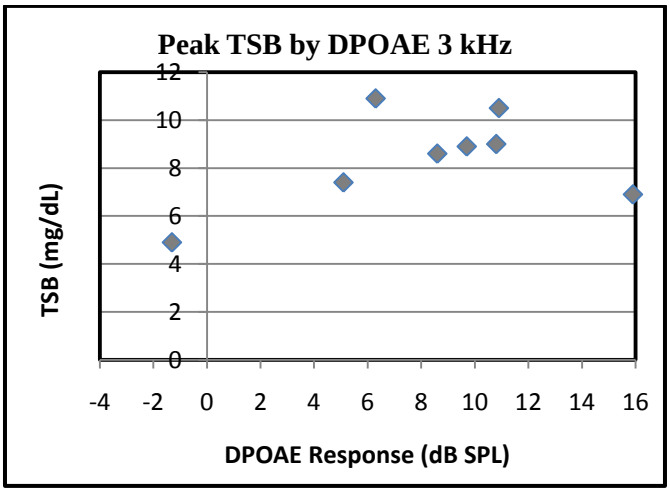


Figure 11 a-c: Scatter plot of peak TSB levels and DPOAE responses from the second follow-up collection for (a) 2 kHz, (b) 3 kHz, and (c) 4 kHz.

(a)



(b)



(c)

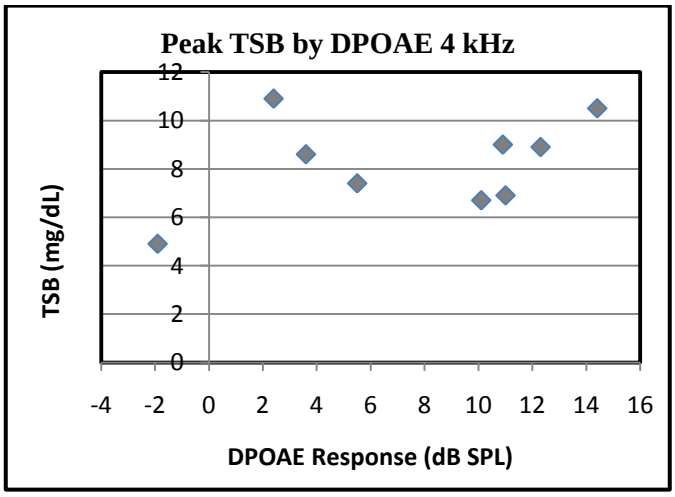
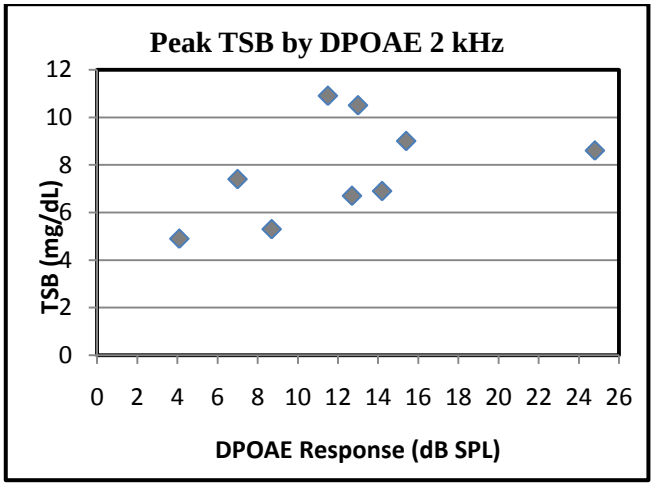
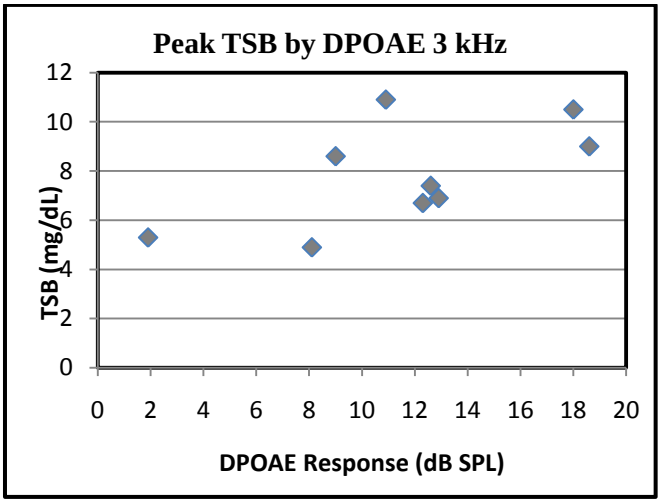


Figure 12 a-c: Scatter plots of peak TSB levels and DPOAE responses from the third follow-up collection for (a) 2 kHz, (b) 3 kHz, and (c) 4 kHz.

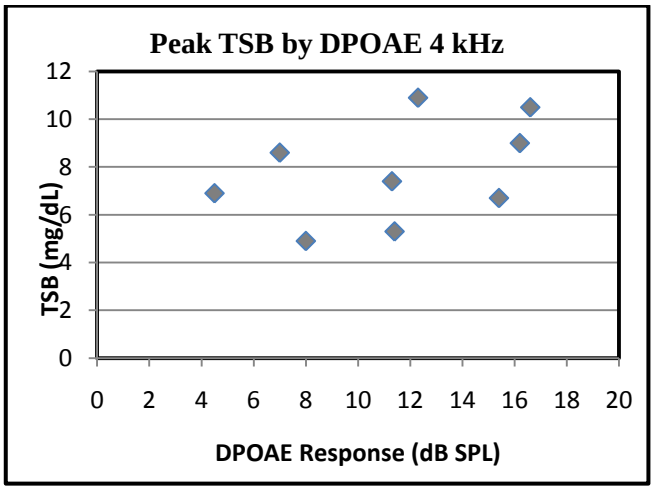
(a)



(b)



(c)



APPENDIX C

HUMAN SUBJECTS COMMITTEE APPROVAL DOCUMENTATION

Recruitment of Patients

The study protocol and informed consent form were submitted to the KUMC Human Subjects Committee (HSC) for review and approval prior to initiation of the study. Recruitment took place from the University of Kansas Hospital NICU. Treating physicians at the KUMC NICU under the direction of Dr. Parimi, co-primary investigator for the study, made referrals. Patients were referred to the Department of Hearing and Speech at KUMC under the direction of Dr. Ferraro, co-primary investigator for the study. Gabriel Bargen, lead clinical investigator for the study, in the KUMC Department of Hearing and Speech, completed subject enrollment into the study, ABR and OAE evaluations, and collected TSB level data. TSB level data was collected following the completion of the ABR/OAE evaluations so the examiner was not biased during the ABR/OAE testing. All ABR tracings and OAE responses were analyzed by the clinical investigator and a blind observer to insure data was as accurate as possible and assessed in a consistent manner to eliminate examiner bias.

Determination of Sample Size

Select previous studies involving hearing loss associated with elevated bilirubin levels have included a wide range of study subjects from eleven to 143 with an average of 62.3 subjects. Rhee, Park, and Jang (1999) included eleven neonates with severe hyperbilirubinemia in their research to clarify the auditory lesion site of hyperbilirubinemic neonates. Kaga, Kitazumi, and Kodama (1979) included 25 babies in their study to show ABRs effectiveness of assessing the

neurotoxic effects of bilirubin on the nervous system. Agrawal et al., (1998) included 30 neonates with and 25 without hyperbilirubinemia in their study to determine the initial ABR abnormalities in babies and the research the possibility of abnormality reversal after therapy. Tan et al. (1992) included 61 children in research to evaluate and compare the factors related to the hearing of 30 neonates with severe hyperbilirubinemia to an age-matched control group of 31. Nakamura and colleagues (1985) included 80 infants in their study to understand the neurotoxicity of neonatal hyperbilirubinemia using ABR. Amin et al (2001) included 143 infants in their study to determine the usefulness of the bilirubin-albumin molar ratio and unbound bilirubin in predicting bilirubin encephalopathy as assessed by ABR. According to a report by Prabhu Parimi (2008), director of the KUMC NICU, approximately 220 infants are admitted annually; of which about 70 are within the 28 to 34 weeks GA range. Based on this information, a sample size of 30 patients was targeted for the study group and 15 patients were targeted for the control group. By having 45 subjects overall we would have had 80% Power to detect a correlation of .41 or larger in magnitude with a type I error rate of 5%.

Informed Consent

Gabriel Bargen, the lead clinical investigator, identifyied potential patients referred by the treating NICU physicians and discussed the advantages, disadvantages, and the amount of time involved for participation in the study with the patient's parent(s). Potential risks and benefits were discussed. If the patient's parent(s) agreed to allow their child to participate in the study then the HSC approved informed consent form was read, discussed and presented for signature by the patient's parent(s) and the principal investigator. The patient's parent(s) received a copy of the signed consent form.

Data Management

Internal monitoring concerning data management and quality control of data were carried out by the clinical investigator and the principal investigators. Data protection was accomplished by KUMC regulations. The KUMC network provides both a network firewall and virus scanning protection from external threats.

Patient confidentiality was ensured by password protected files and patient charts where applicable. Case Report Forms (CRF) and study-specific source documents (not to include the official Kansas University Hospital Medical Record) for each subject were stored in a study folder located in the Department of Hearing and Speech Audiology Clinic. The study folder was stored in locked file cabinet. An ID number identified all subjects' paper records and all other applicable files.

The clinical investigator and principal investigators had access to the locked cabinets. Subjects' study numbers and associated demographic data were available in the medical record, which included: sex, race, date of birth, date of study visits, and study number.

All patients assigned to the study were entered into a logbook. The logbook was kept by the clinical investigator in the Hearing and Speech Department. After study inclusion, each patient was entered into their own CRF. Data entered into the CRF included demographic information, TBS levels, and any adverse events. Any corrections to the CRF were made in ink by drawing a single line through the incorrect data, making the correction, dating the correction, and providing initials of the person making the change. Every possible effort was made to maintain confidentiality of study data in any form. Given that resources were used to support this investigation, the data were subject to audit by the HSC.

At each follow-up data collection the investigator reported on the occurrance of any adverse events since the last visit as reported by the patients medical record and bedside chart. Information collected for the study was demographic information including risk factors for hearing loss, TBS levels, and results from the ABR and OAE evaluations. The data were obtained specifically for research purposes.

Protocol Deviations

No protocol deviations occurred during the completion of this research project. To account for the majority of missing data during data analysis, an independent consulting statistician used methods warranted to account for missing data. One subject was discharged prior to completing data collection for the study. No additional data were collected post discharge. Data collected up until the point of subject discharge and subsequent withdrawl from the study were used in data analysis for the study.

APPENDIX D

RAW DATA

Table D1: ABR absolute latencies (msec) of waves I, III, and V at 80 dB nHL. Means ± standard deviations are provided at bottom of table. GA, birth weight, and peak TSB are included for reference.

Subject	GA	Birth weight	Peak TSB	Ear	Baseline			1st Follow up			2nd Follow up			3rd Follow up		
Absolute Latency (ms)					I	III	V	I	III	V	I	III	V	I	III	V
1	33	1830	10.9	R	1.83	5.26	8.64				1.95	5.01	7.82	1.76	4.64	7.51
				L				1.76	5.14	8.45	1.70	4.82	7.82	1.70	4.45	7.14
2	33	1610	9	R										1.70	4.14	6.82
				L	1.70	5.01	8.14							1.70	4.39	6.70
3	34	1930	8.9	R		4.51	6.95				1.80	4.45	6.76			
				L	1.89	4.82	7.32				1.95	4.51	6.95			
4	34	1930	4.9	R	1.76	4.51	7.01					4.26	6.76	1.64	4.39	6.64
				L				2.00	4.45	6.70	2.20	4.64	6.82	1.51	4.26	6.51
5	28	1060	8.6	R	2.01	4.76	8.82				2.08	5.14	8.32	1.89	4.57	7.39
				L							1.76	4.89	7.82	1.89	4.64	7.26
6	30	1210	5.3	R							1.89	4.64	7.64	1.95	4.32	7.20
				L	2.02	5.20	7.07	2.08	4.95	6.70	1.64	4.45	7.64	1.70	4.26	7.14
7	29	1600	6.7	R				1.76	4.95	6.76	1.89	4.76	7.82	1.64	4.26	7.70
				L				1.76	4.80	6.32	1.64	4.39	6.26	1.51	4.14	7.20
8	32	2113	10.5	R										1.70	4.51	7.76
				L							1.58	4.20	7.39	1.85	4.51	6.95
9	31	1800	6.9	R				2.01	5.07	8.45	1.83	4.57	7.39	1.89	4.57	7.57
				L	1.95	5.20	8.70	1.95	5.01	6.95	1.76	4.70	7.82	1.64	4.76	7.32
10	31	1480	7.4	R				1.76	4.32	7.07				2.85	5.39	7.57
				L	1.83	4.51	7.07	1.83	4.51	7.45	1.89	4.39	6.95	1.58	4.51	6.82
m	31.5	1656.3	7.91		1.87	4.86	7.75	1.88	4.80	7.21	1.84	4.61	7.37	1.78	4.48	7.18
SD	2.07	333.40	2.03		0.12	0.31	0.81	0.13	0.30	0.77	0.17	0.26	0.56	0.30	0.29	0.37

Table D2: ABR latencies (msec) for I-III, III-V, and I-V interwave intervals measured at 80 dB nHL. Means ± standard deviations are provided at the bottom of the table. GA, birth weight, and peak TSB are included for reference.

Subject	GA	Birth weight	Peak TSB	Ear	Baseline			1st Follow up			2nd Follow up			3rd Follow up		
Interwave Interval (ms)					I-III	III-V	I-V	I-III	III-V	I-V	I-III	III-V	I-V	I-III	III-V	I-V
1	33	1830	10.9	R	3.44	3.38	6.81				3.12	3.00	6.13	2.87	2.88	5.75
				L				3.38	3.31	6.69	3.06	2.81	5.88	2.75	2.69	5.44
2	33	1610	9	R										2.44	2.69	5.13
				L	3.31	3.13	6.44							2.69	2.31	5.00
3	34	1930	8.9	R		2.44					2.65	2.31	4.96			
				L	2.94	2.50	5.44				2.56	2.44	5.00			
4	34	1930	4.9	R	2.75	2.50	5.25					2.50		2.75	2.25	5.00
				L				2.45	2.25	4.70	2.44	2.19	4.63	2.75	2.25	5.00
5	28	1060	8.6	R	2.75	4.06	6.81				3.06	3.19	6.25	2.69	2.81	5.50
				L							3.12	2.94	6.06	2.75	2.63	5.38
6	30	1210	5.3	R							2.75	3.00	5.75	2.37	2.88	5.25
				L	3.18	1.88	5.05	2.87	1.75	4.63	2.81	3.19	6.00	2.56	2.88	5.44
7	29	1600	6.7	R				3.19	1.81	5.00	2.87	3.06	5.93	2.62	3.44	6.06
				L				3.04	1.52	4.56	2.75	1.88	4.63	2.62	3.06	5.69
8	32	2113	10.5	R										2.81	3.25	6.06
				L							2.62	3.19	5.81	2.66	2.44	5.10
9	31	1800	6.9	R				3.06	3.38	6.44	2.75	2.81	5.56	2.69	3.00	5.69
				L	3.25	3.50	6.75	3.06	1.94	5.00	2.94	3.13	6.06	3.12	2.56	5.69
10	31	1480	7.4	R				2.56	2.75	5.31				2.54	2.19	4.72
				L	2.69	2.56	5.25	2.69	2.94	5.63	2.50	2.56	5.06	2.93	2.31	5.24
m	31.5	1656.3	7.91		3.04	2.88	5.98	2.92	2.41	5.33	2.80	2.76	5.58	2.70	2.70	5.40
SD	2.07	333.40	2.03		0.29	0.68	0.79	0.30	0.71	0.78	0.22	0.40	0.57	0.17	0.36	0.38

Table D3: ABR thresholds (dB nHL) at each data collection. Means ± standard deviations are provided at the bottom of the table. GA and peak TSB are included for reference.

Subject	GA	Peak TSB	Ear	Baseline Threshold	1st Follow-up Threshold	2nd Follow-up Threshold	3rd Follow-up Threshold
1	33	10.9	R	18	23	17.5	12.5
			L		23	12.5	12.5
2	33	9.0	R				17.5
			L	22.5			17.5
3	34	8.9	R			12.5	
			L	17.5		12.5	
4	34	4.9	R	17.5		12.5	17.5
			L		17.5	12.5	17.5
5	28	8.6	R			12.5	7.5
			L			17.5	17.5
6	30	5.3	R			17.5	17.5
			L	17.5	17.5	22.5	7.5
7	29	6.7	R		17.5	27.5	17.5
			L		17.5	17.5	22.5
8	32	10.5	R				17.5
			L			17.5	25
9	31	6.9	R		17.5	12.5	22.5
			L	37.5	17.5	22.5	27.5
10	31	7.4	R		22.5		17.5
			L	17.5	17.5	12.5	17.5
M	31.5	7.91		21.07	19.10	16.25	17.36
SD	2.07	2.03		7.48	2.58	4.65	5.18

Table D4: DPOAE level and level of the noise floor in dB SPL from the baseline and first follow-up collections. GA and peak TSB are included for reference.

Subject	GA	Peak TSB	Ear	Baseline						1st Follow up					
				2kHz		3kHz		4kHz		2kHz		3kHz		4kHz	
				DP	NF	DP	NF	DP	NF	DP	NF	DP	NF	DP	NF
1	33	10.9	R	**7.8**	**9.9**	**6.0**	**8.3**	11.6	4.9	9.5	-5.7	7.9	-.8	6.2	-2
			L			**3.4**	**3.6**	14.7	1.8	13	2.9	13.4	5.2	13.1	3.1
2	33	9	R			9.6	-.2	8.8	-.7	9.3	-.1	12.6	4.5	5.4	-6
			L			7.8	-.7	8.8	-.2	10.3	-2.6	7.1	-1.8	5.4	-2.9
3	34	8.9	R	7.6	1.3	**6.6**	**6**	**1.3**	**-3.4**	7.5	-.8	3.3	-7	**-2.8**	**-6.9**
			L	1.2	-7.4	4.5	-12.4	7.8	-15.2	8	-11.6	7.6	-6	8.7	-10.9
4	34	4.9	R	**6.3**	**2.4**	8.5	-.9	**-2.7**	**-4.2**	11.9	2.5	11.8	3.8	9.5	1.4
			L	**.3**	**-1.3**	**6.1**	**2.7**	8.6	-1.2	3.3	-14.4	-1.69	-12.4	.3	-10.7
5	28	8.6	R	8.5	-5	**-6.8**	**-.7**	**-4.7**	**-4.8**	**14.7**	**9.7**	23.3	12.8	**-2.1**	**-1.1**
			L							17	7.7	**5.9**	**7**	**11.3**	**10.6**
6	30	5.3	R												
			L												
7	29	6.7	R	**11.6**	**8.6**	5.4	-4.7	11.9	.8	5.8	-3.1	5.9	-3.4	8.7	-4.5
			L	5.1	-7.5	5.5	-10.6	10.3	-10	4.1	-11	5.2	-5.6	8.7	-2.2
8	32	10.5	R												
			L	4.5	-8.6	10.5	-8.8	9.3	-10.8						
9	31	6.9	R							13	3.5	7.9	-2.3	7.2	-5
			L	**8.5**	**6.9**	14.4	5.3	5.9	-4	10.9	-3.4	7.5	-5	-.7	-12.6
10	31	7.4	R							12.3	3.1	9	-7.2	18.1	-4.5
			L	4.7	-5	-5.1	-12.9	-2.4	-8.7	6.1	-2.4	8.6	.5	5.9	-6

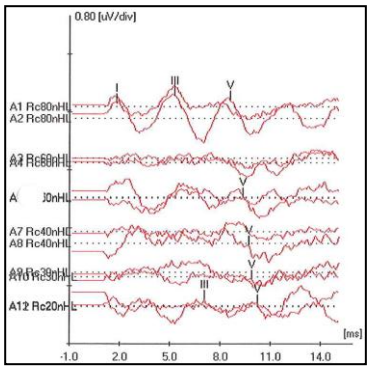
Bold = SNR < 6 dB therefore the measurement not used in the analysis

Table D5: DPOAE level and level of the noise floor in dB SPL from the second and third follow-up collections. GA and peak TSB are included for reference.

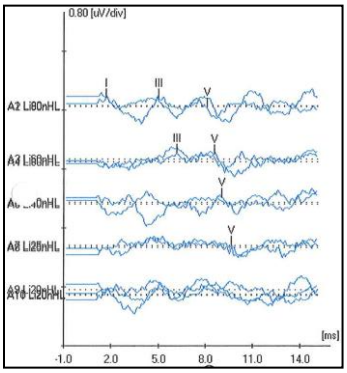
Subject	GA	Peak TSB	Ear	2nd Follow up						3rd Follow up					
				2kHz		3kHz		4kHz		2kHz		3kHz		4kHz	
				DP	NF	DP	NF	DP	NF	DP	NF	DP	NF	DP	NF
1	33	10.9	R	2.3	-6.2	6.3	-8.7	2.4	-8.1	11.5	2	10.9	1.4	12.3	-3.8
			L	8.3	-.3	8.8	-4.8	6.5	-4	6.1	-4.5	9.5	1.3	7.7	-4.4
2	33	9	R							15.4	1.4	18.6	3.1	16.2	.3
			L	12.9	2	10.8	-4.3	10.9	-6.1	12.6	3.8	12.5	1.6	8.6	-1.5
3	34	8.9	R	8.4	0	5.1	-2.7	17.4	6						
			L	13	-7.8	9.7	-11.4	12.3	-17.2						
4	34	4.9	R	2.2	-9.7	3.4	-8.7	3.8	-7.3	4.1	-2.8	8.1	-1.3	8	-1.6
			L	**-3.9**	**-7.6**	-1.3	-11.1	-1.9	-10.5	.8	-15.4	4.2	-14.6	5.5	-7.8
5	28	8.6	R	**5**	**-2.2**	**.3**	**-5.3**	4.5	-4.3	**5.7**	**3.4**	**3.3**	**3**	12.1	3.2
			L	**7.3**	**10.5**	8.6	-1	3.6	-6.5	24.8	16.2	9	-4.5	7	-1.6
6	30	5.3	R	**11.5**	**8.6**	**4.7**	**12.7**	10.4	-.5	8.7	1.3	1.9	-6.9	11.4	2.1
			L					**2.5**	**.8**	7.9	-3.4	4.1	-4.2	8.8	-5
7	29	6.7	R	**6.3**	**3.1**	**-.5**	**-6**	10.1	2	12.6	1.1	12.1	1.9	14.7	-.3
			L	12.5	5.7	9	2.2	12.1	-3.5	12.7	4.1	12.3	2.1	15.4	4.8
8	32	10.5	R	11.7	3.4	9.4	.5	13.9	-5.8	18.1	-6.7	16.9	-9	17.7	-8
			L	9.6	-2.7	10.9	-5.9	14.4	-9.9	13	-.9	18	-9.9	16.6	-7.3
9	31	6.9	R	15	6.2	12.4	0	10.3	-2.8	2.5	-6.5	14.1	5.8	18.9	.3
			L	11.6	-5.7	15.9	-7.2	11	-6.8	14.2	.6	12.9	2.7	4.5	-9.6
10	31	7.4	R	3.8	-5.2	7.7	-4.2	16.5	-9.5	7	-2	12.6	4.4	11.3	-2
			L	**7.9**	**8.6**	5.1	-3.6	5.5	-5	20.3	12.0	9.1	-0.1	11.8	2.1

Bold = SNR < 6 dB therefore measurement was not used in the analysis

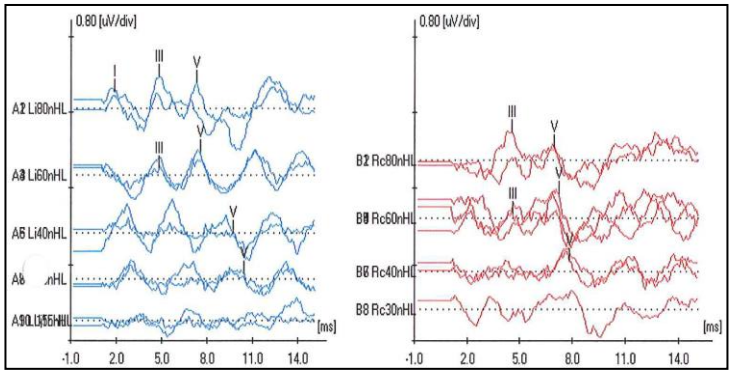
Figure D1 a-h: ABR tracings from baseline collection. ABR response (dB nHL) as a function of time (msec).



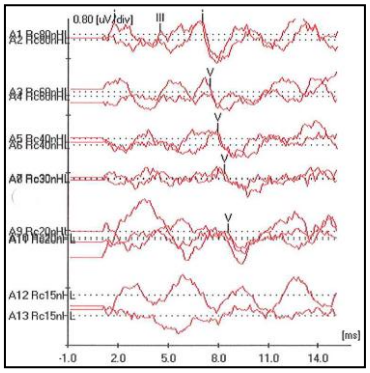
(a) Subject 1 – right ear



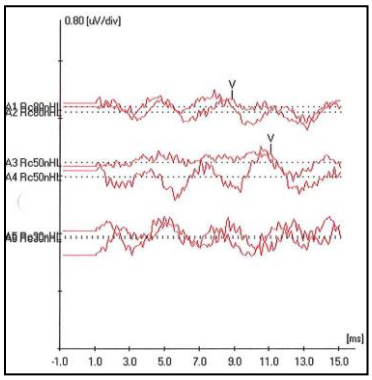
(b) Subject 2 – left ear



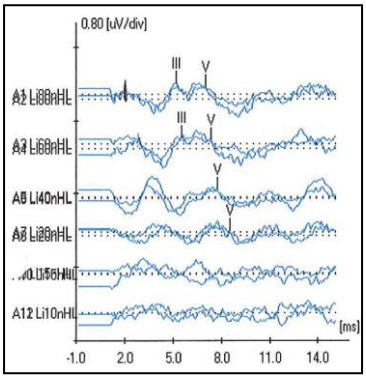
(c) Subject 3 – left and right ear



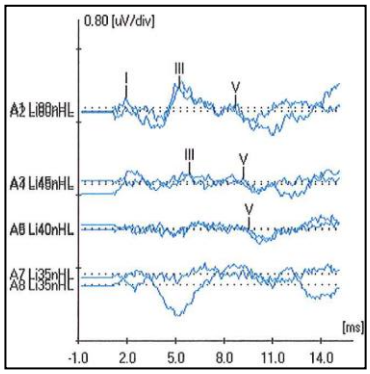
(d) Subject 4 – right ear



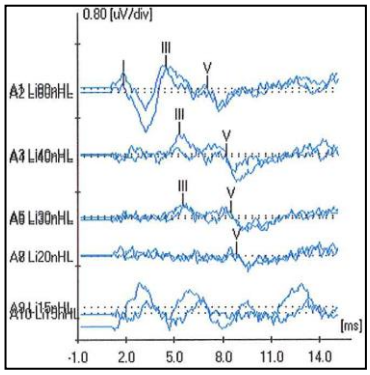
(e) Subject 5 - right ear



(f) Subject 6 – left ear

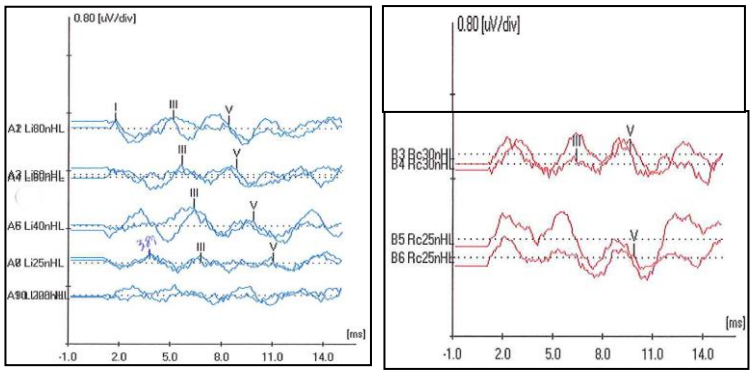


(g) Subject 9 – left ear

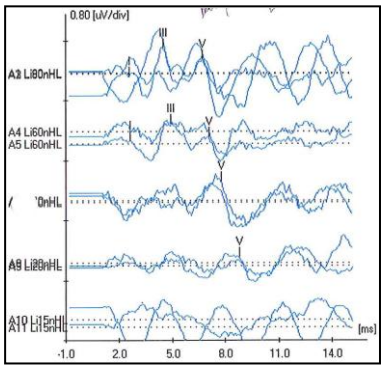


(h) Subject 10 – left ear

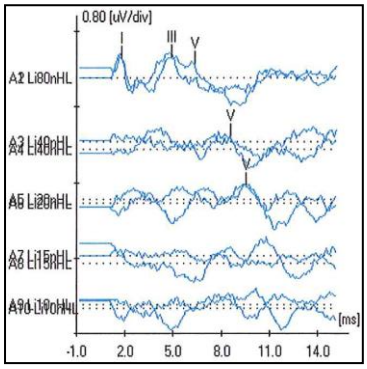
Figure D2 a-f: ABR tracings from first follow-up collection. ABR response (dB nHL) as a function of time (msec).



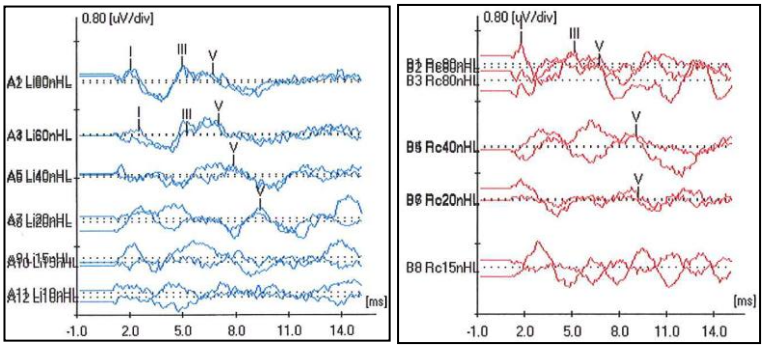
(a) Subject 1 – left and right ear



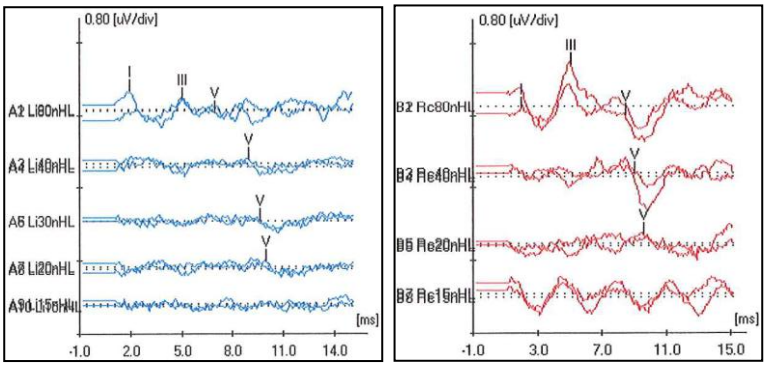
(b) Subject 4 – left ear



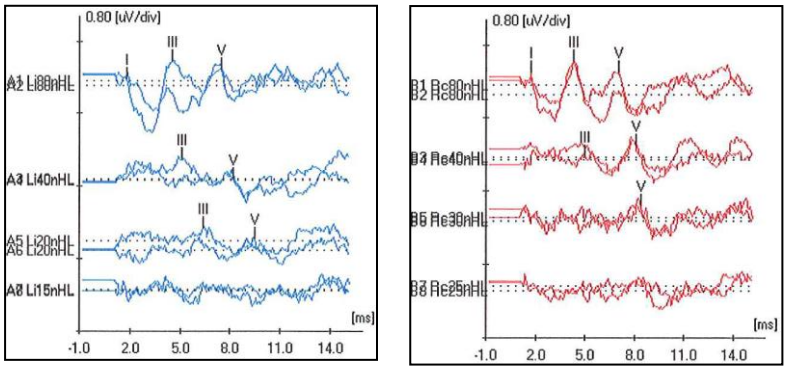
(c) Subject 6 – left ear



(d) Subject 7 – left and right ear

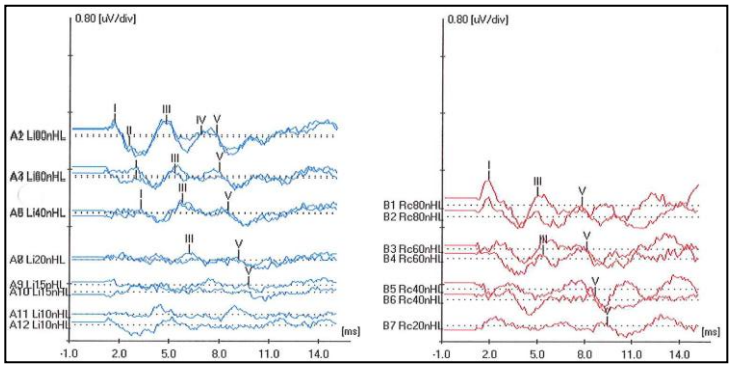


(e) Subject 9 – left and right ear

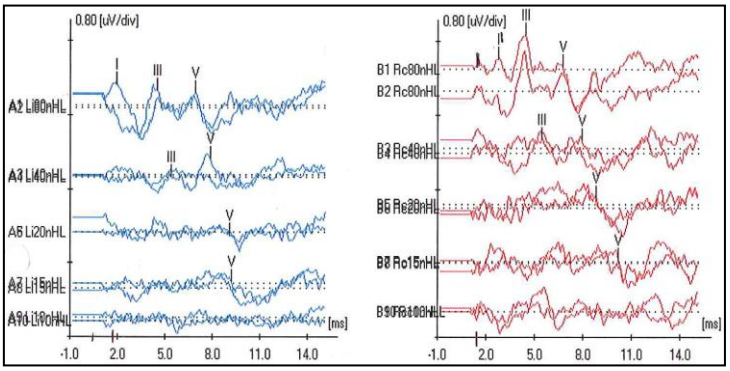


(f) Subject 10 – left and right ear

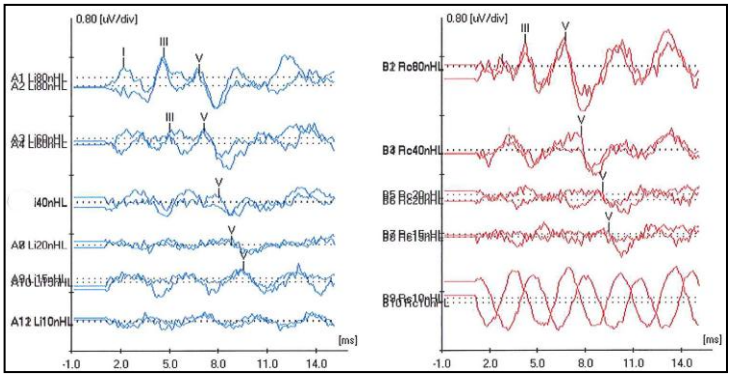
Figure D3 a-i: ABR tracings from second follow-up collection. ABR response (dB nHL) as a function of time (msec).



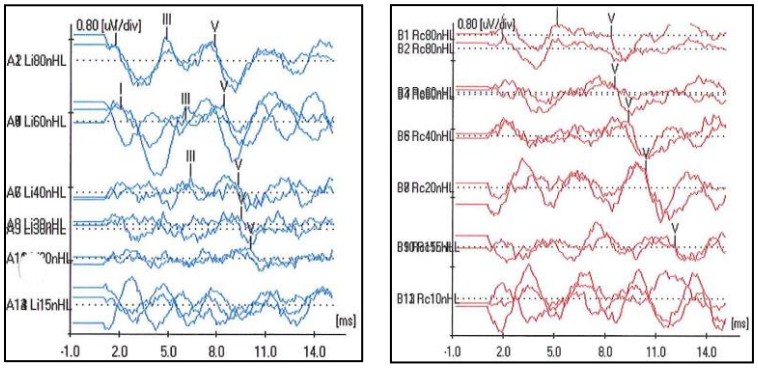
(a) Subject 1 – left and right ear



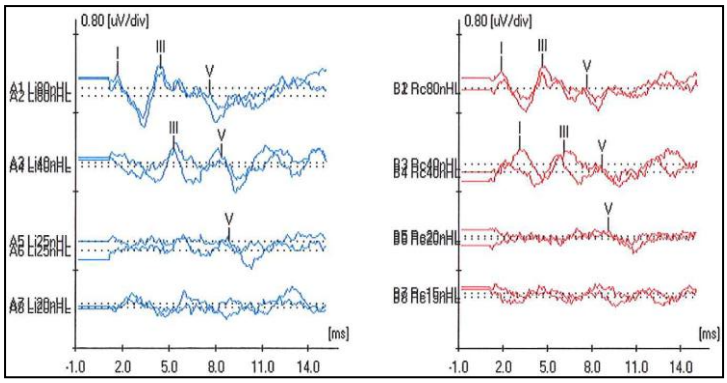
(b) Subject 3 – left and right ear



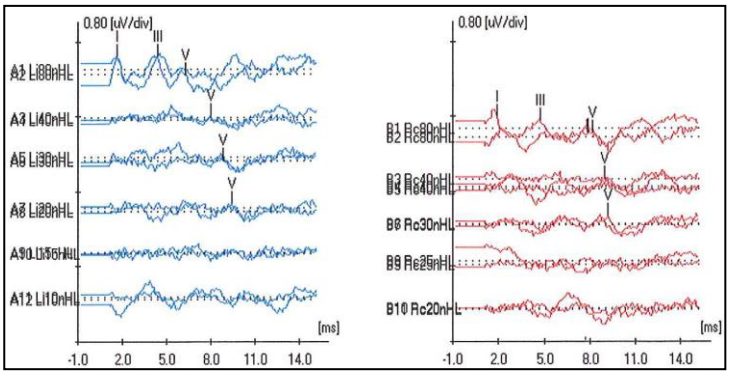
(c) Subject 4 – left and right ear



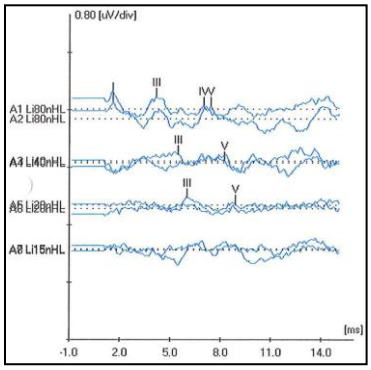
(d) Subject 5 – left and right ear



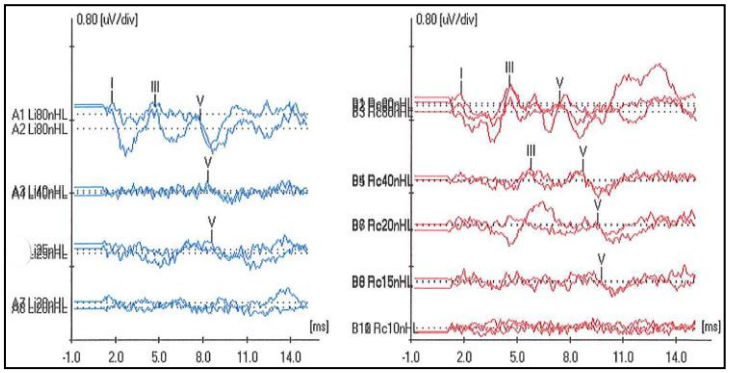
(e) Subject 6 – left and right ear



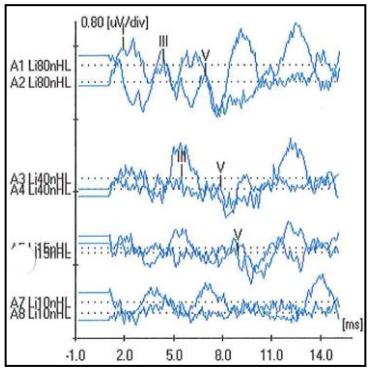
(f) Subject 7 – left and right ear



(g) Subject 8 – left ear

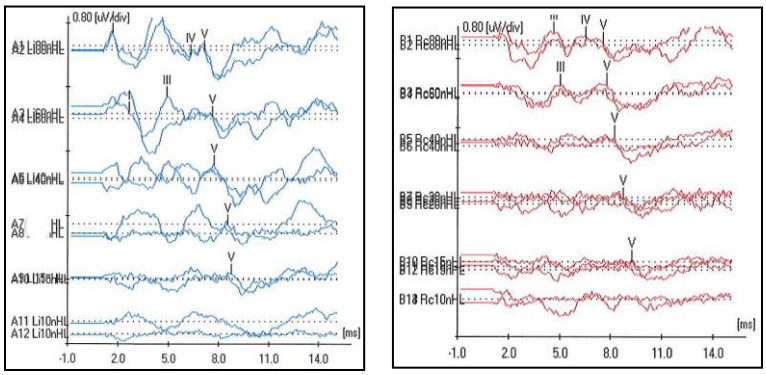


(h) Subject 9 – left and right ear

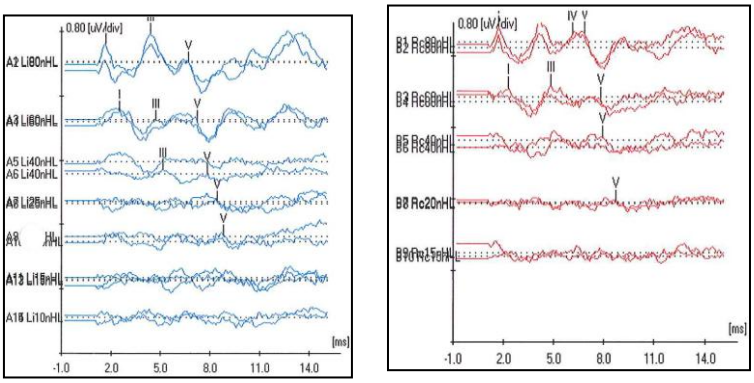


(i) Subject 10 – left ear

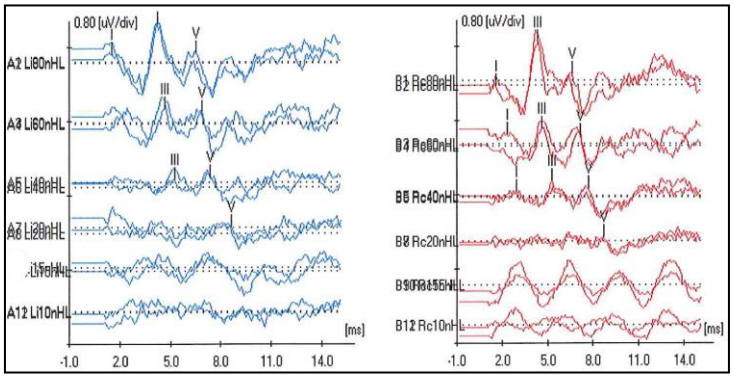
Figure D4 a-i: ABR tracings from third follow-up collection. ABR response (dB nHL) as a function of time (msec).



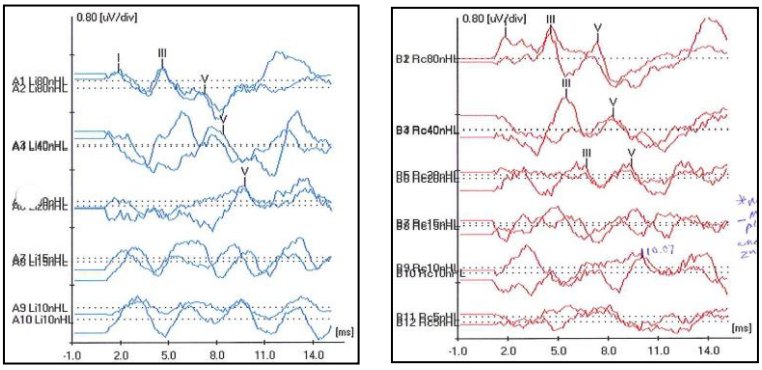
(a) Subject 1 – left and right ear



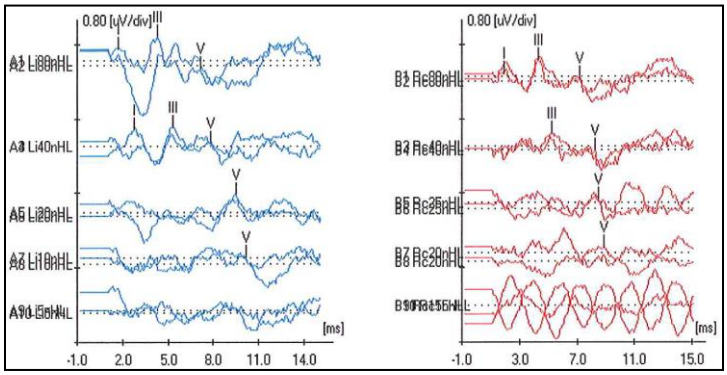
(b) Subject 2 – left and right ear



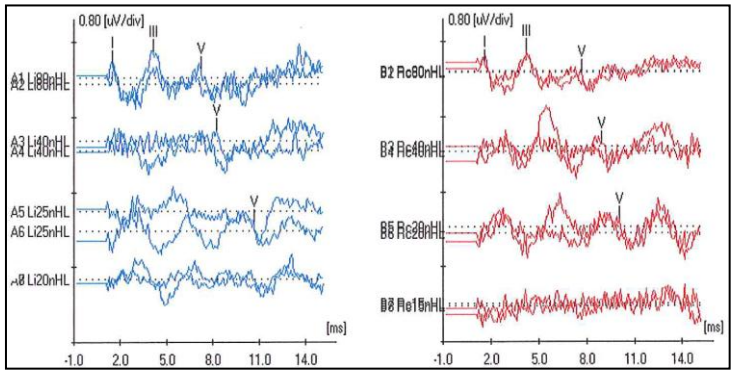
(c) Subject 4 – left and right ear



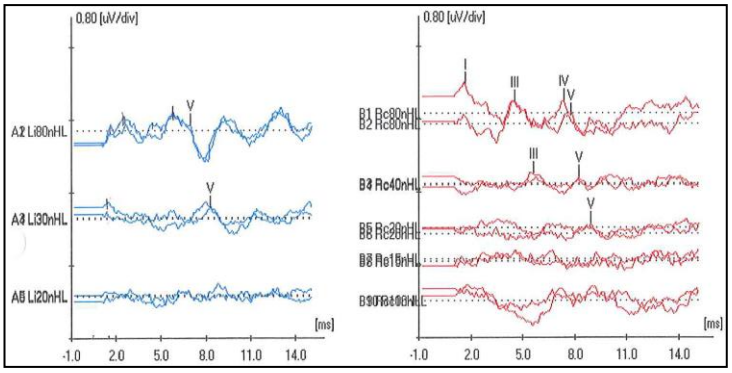
(d) Subject 5 – left and right ear



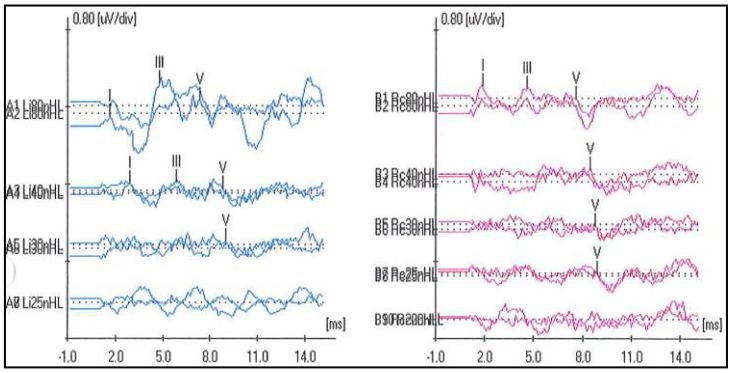
(e) Subject 6 – left and right ear



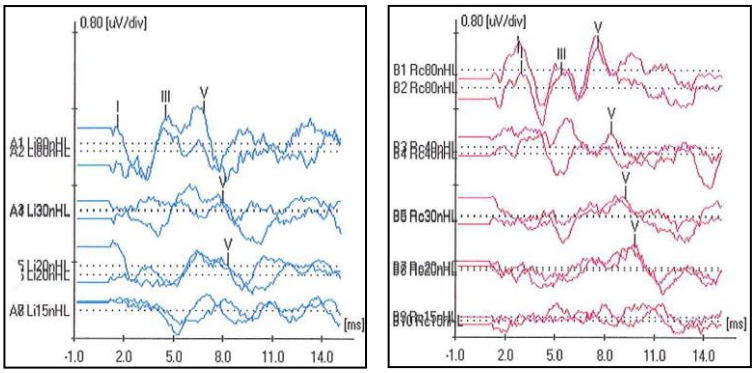
(f) Subject 7 – left and right ear



(g) Subject 8 – left and right ear

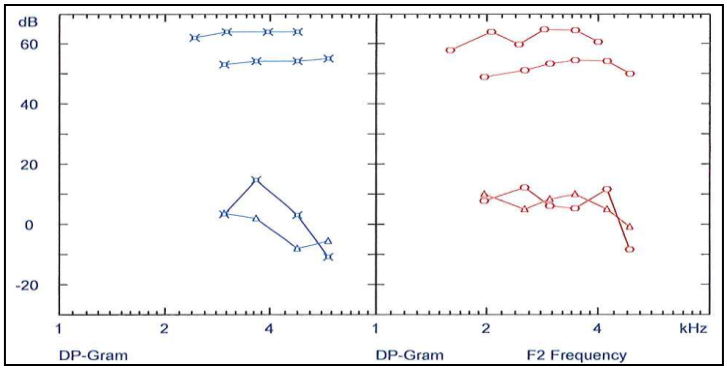


(h) Subject 9 – left and right ear

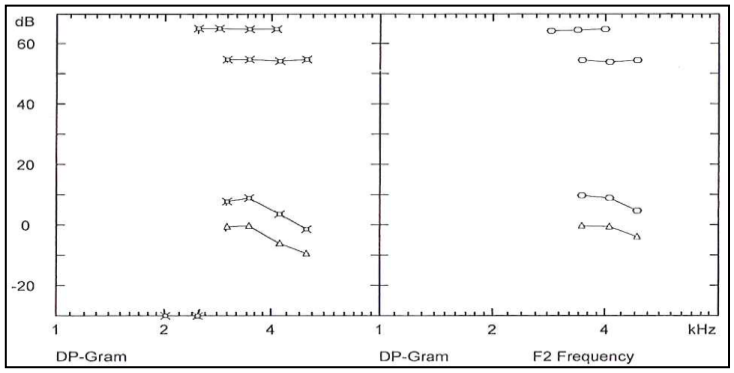


(i) Subject 10 – left and right ear

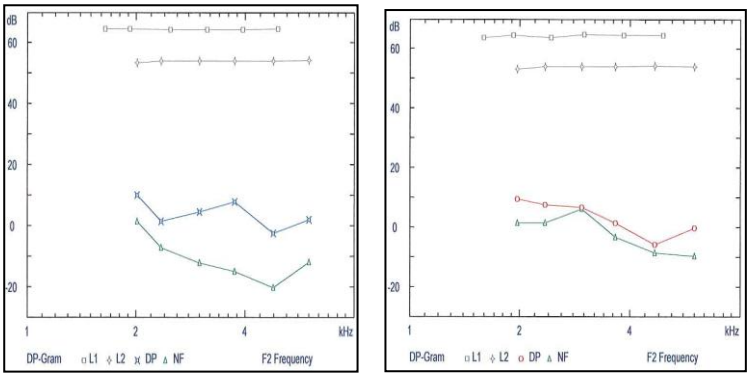
Figure D5 a-i: DP-grams from baseline collection. DPOAE level (dB SPL) as a function of f_2 frequency (Hz).



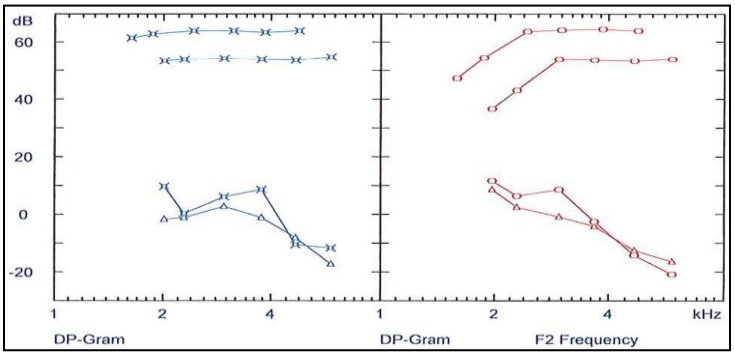
(a) Subject 1 – left and right ear



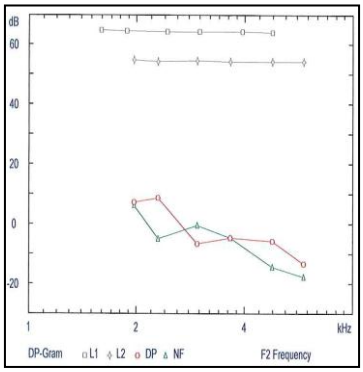
(b) Subject 2 – left and right ear



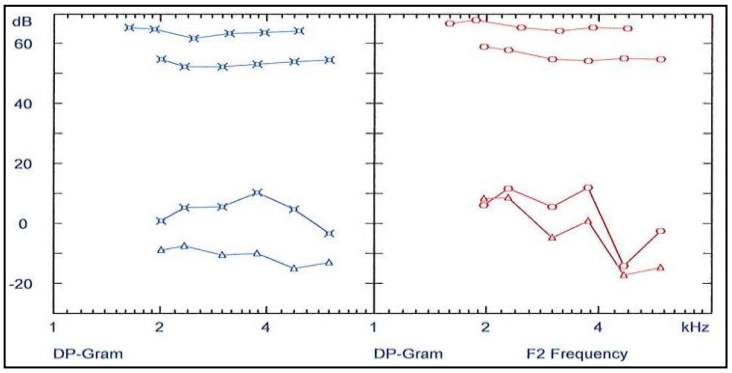
(c) Subject 3 – left and right ear



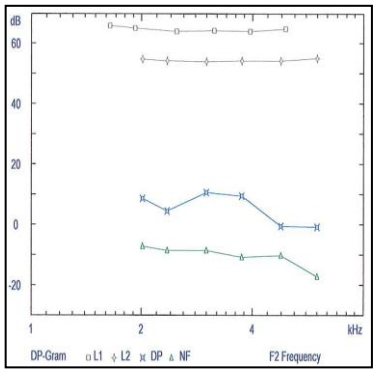
(d) Subject 4 – left and right ear



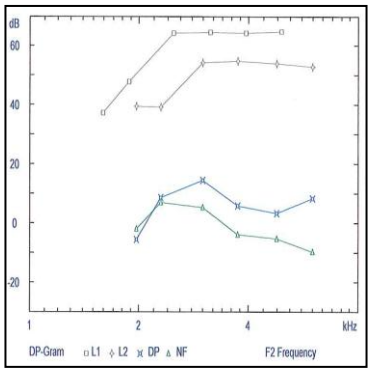
(e) Subject 5 – right ear



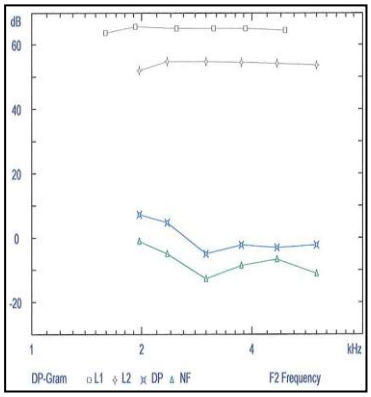
(f) Subject 7 – left and right ear



(g) Subject 8 – left ear

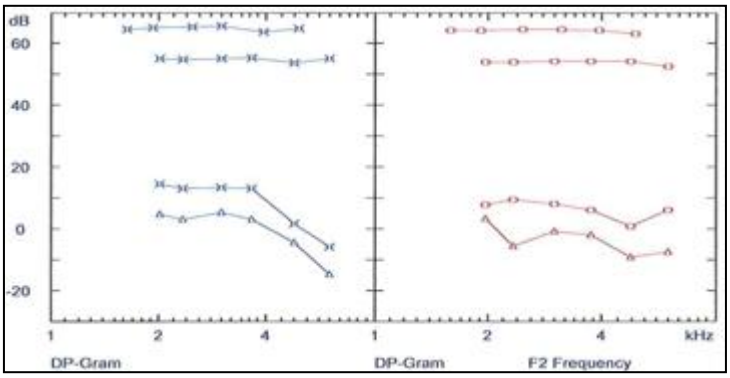


(h) Subject 9 – left ear

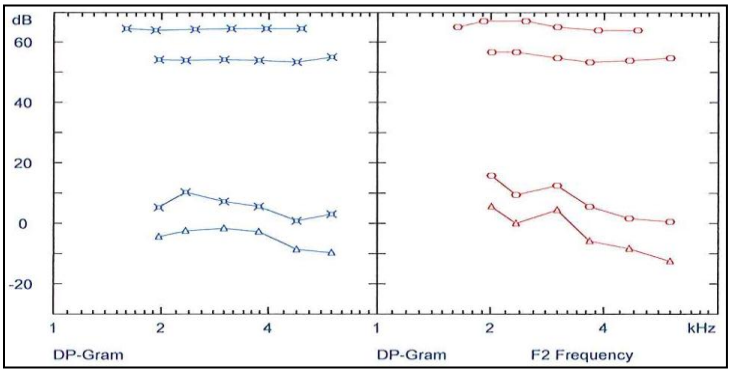


(i) Subject 10 – left ear

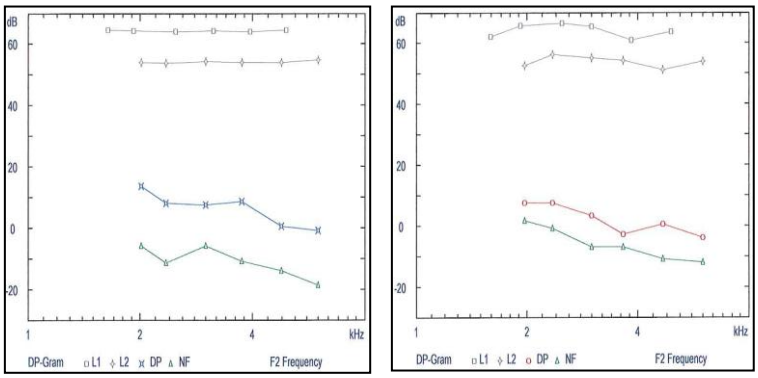
Figure D6 a-h: DP-grams from first follow-up collection. DPOAE level (dB SPL) as a function of f_2 frequency (Hz).



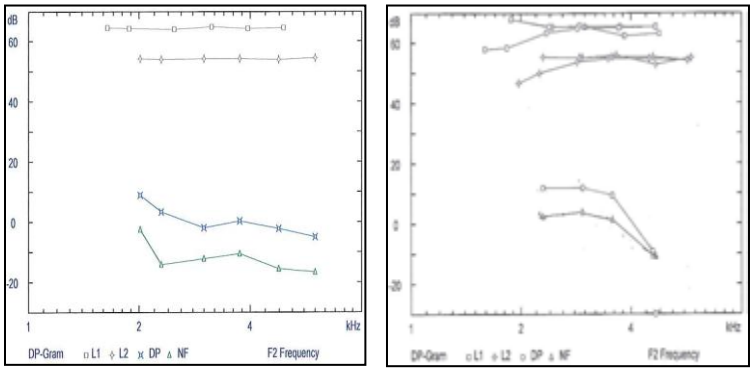
(a) Subject 1 – left and right ear



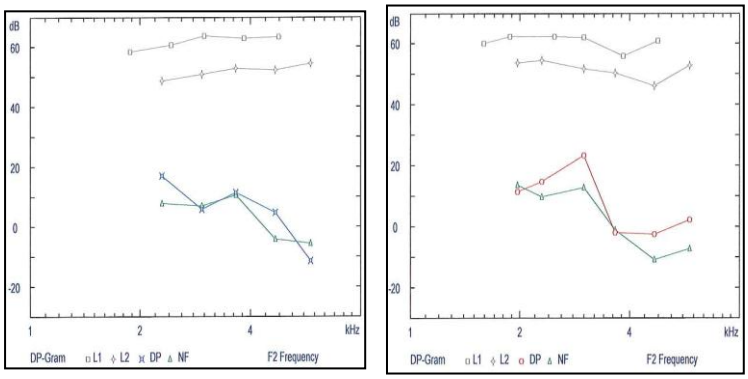
(b) Subject 2 – left and right ear



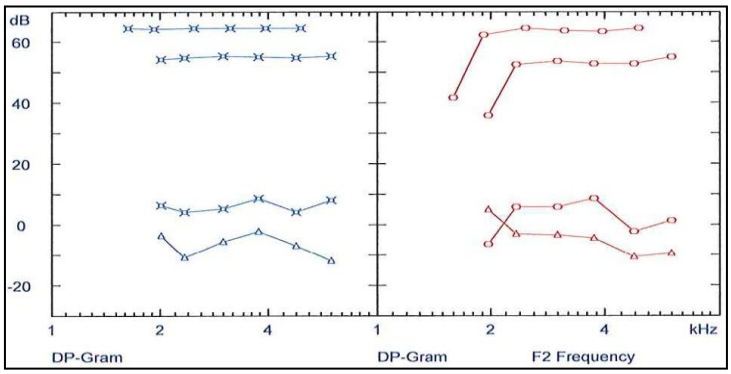
(c) Subject 3 – left and right ear



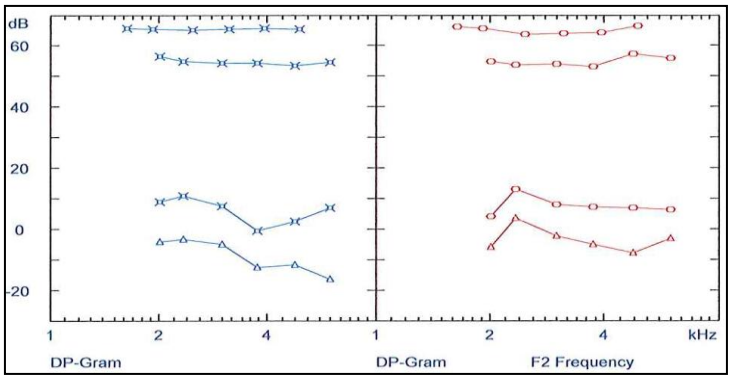
(d) Subject 4 – left and right ear



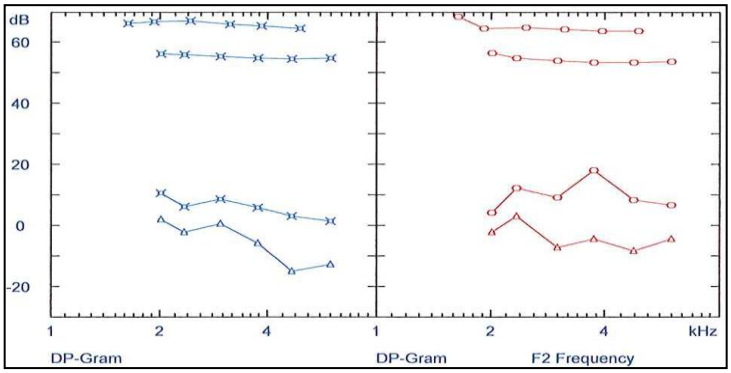
(e) Subject 5 – left and right ear



(f) Subject 7 – left and right ear

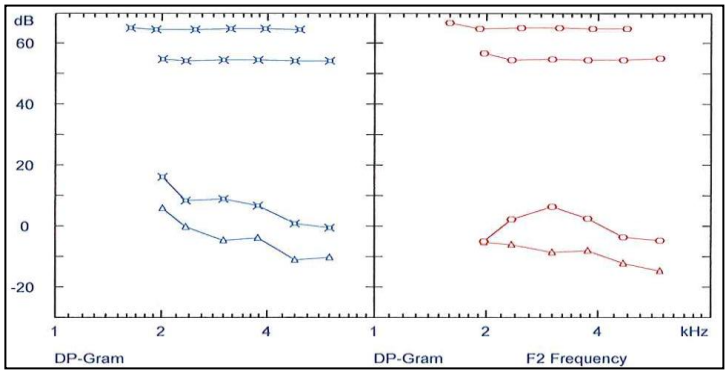


(g) Subject 9 – left and right ear

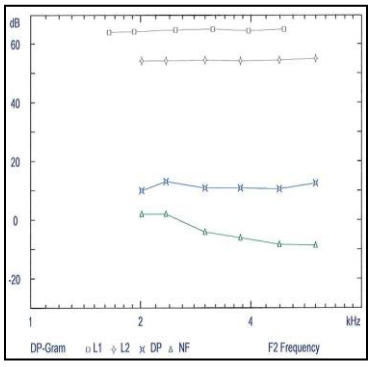


(h) Subject 10 – left and right ear

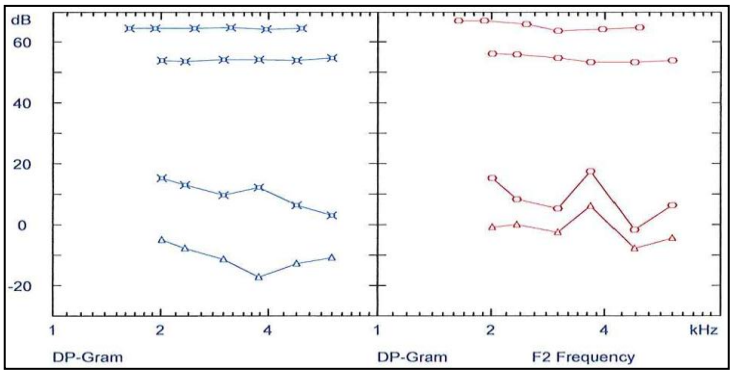
Figure D7 a-j: DP-grams from second follow-up collection. DPOAE level (dB SPL) as a function of f_2 frequency (Hz).



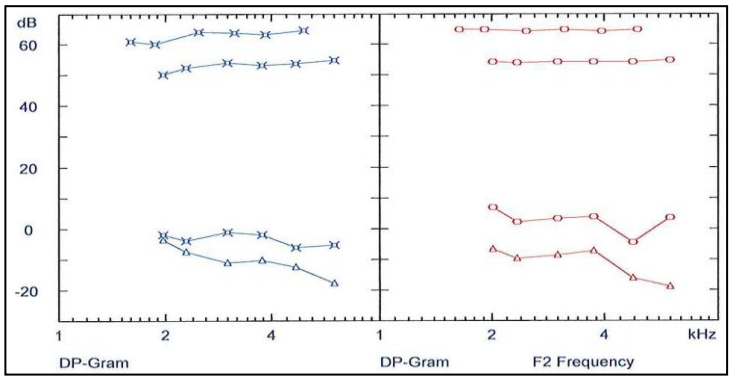
(a) Subject 1 – left and right ear



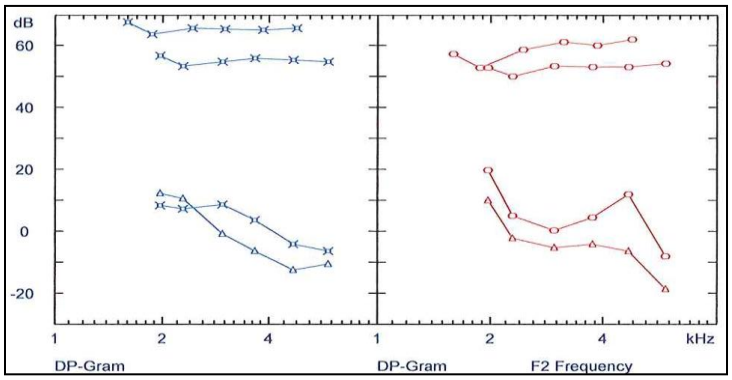
(b) Subject 2 – left ear



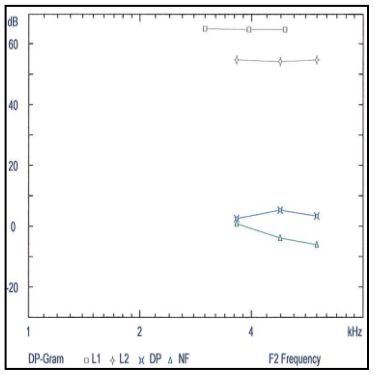
(c) Subject 3 – left and right ear



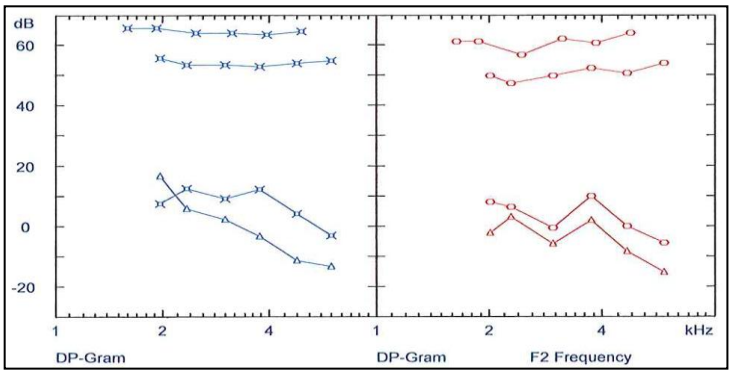
(d) Subject 4 – left and right ear



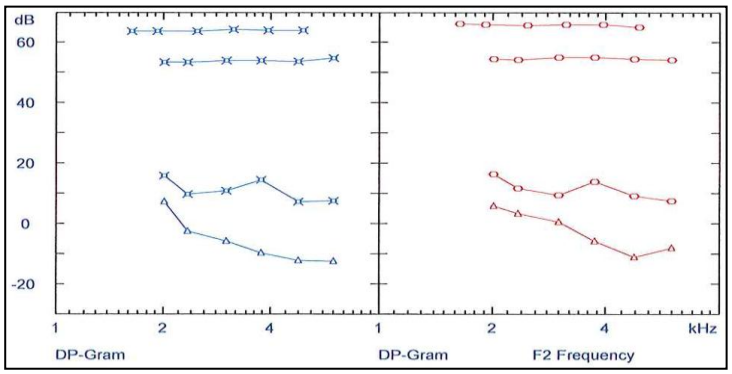
(e) Subject 5 – left and right ear



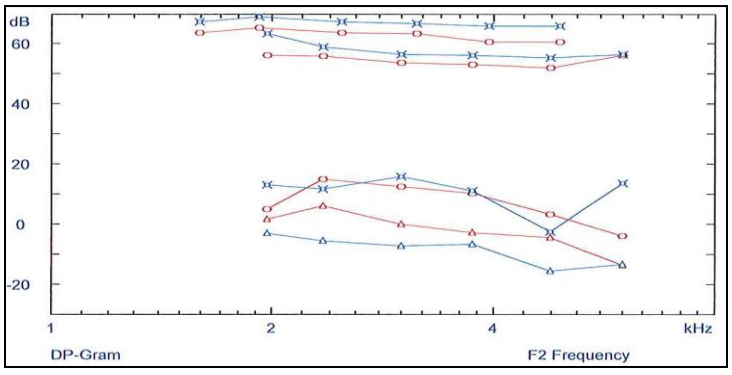
(f) Subject 6 – left ear



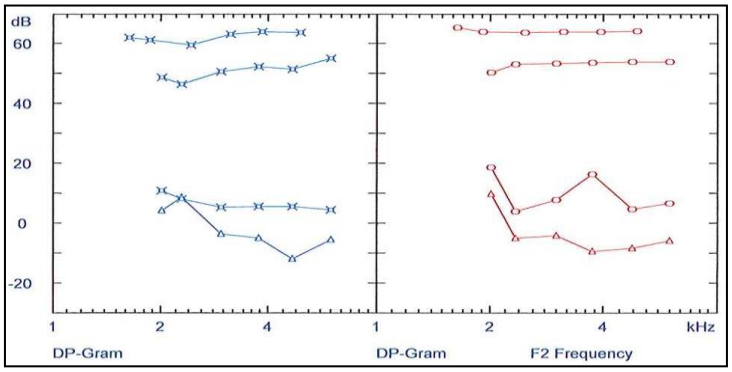
(g) Subject 7 – left and right ear



(h) Subject 8 – left and right ear

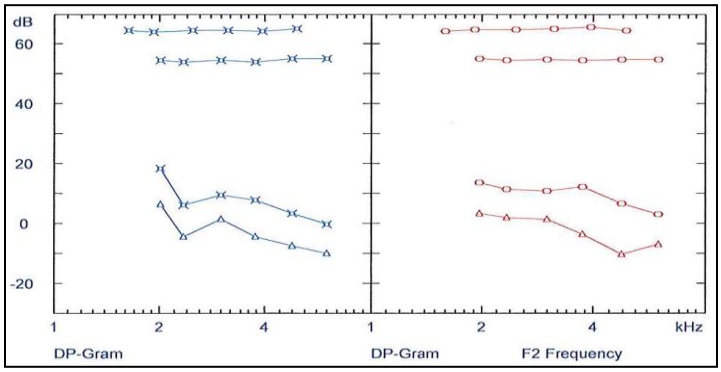


(i) Subject 9 – left and right ear (superimposed)

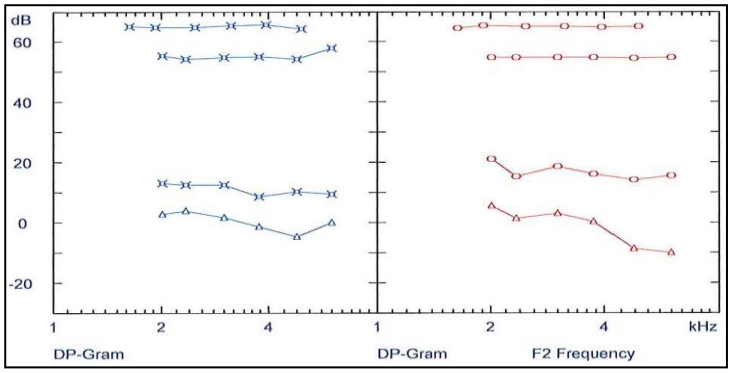


(j)Subject 10 – left and right ear

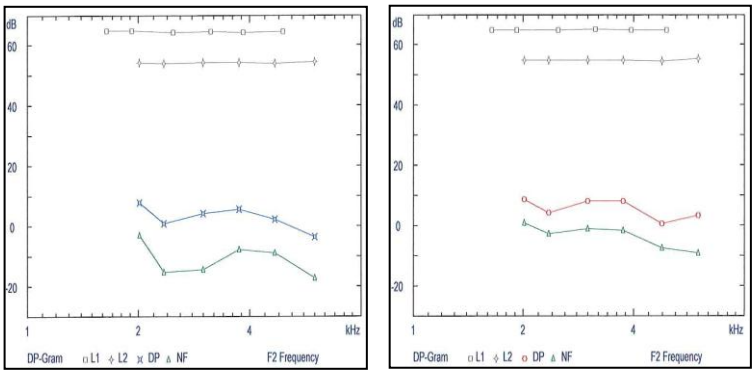
Figure D8 a-i: DP-grams from third follow-up collection. DPOAE level (dB SPL) as a function of f_2 frequency (Hz).



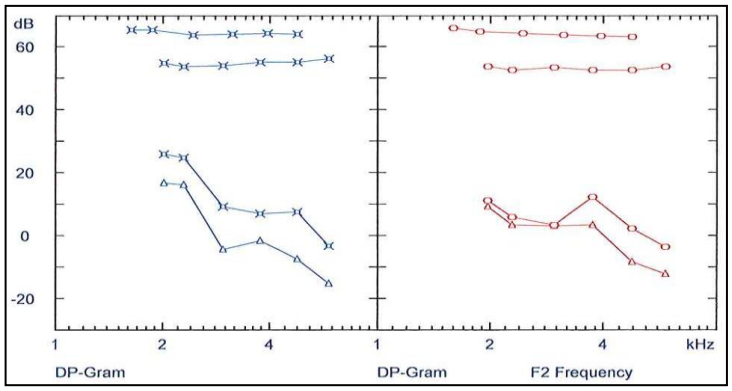
(a) Subject 1 – left and right ear



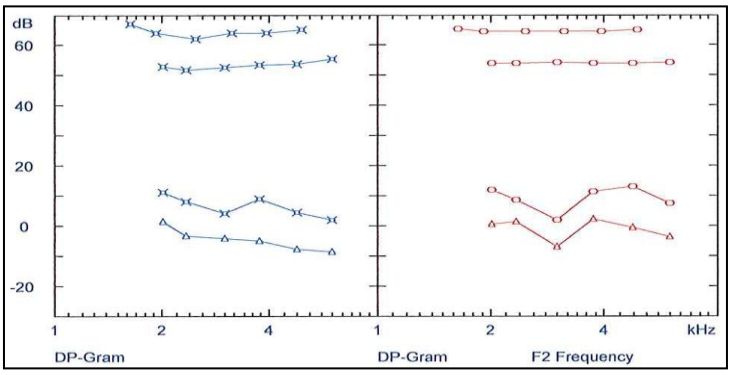
(b) Subject 2 – left and right ear



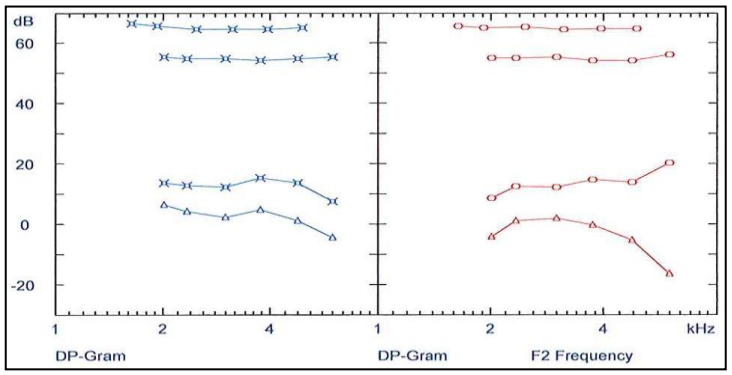
(c) Subject 4 – left and right ear



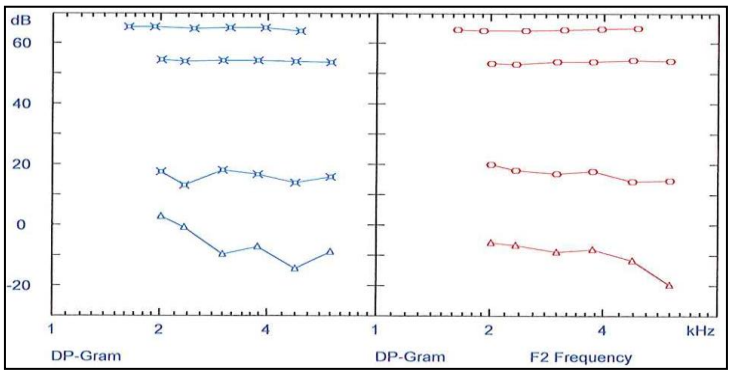
(d) Subject 5 – left and right ear



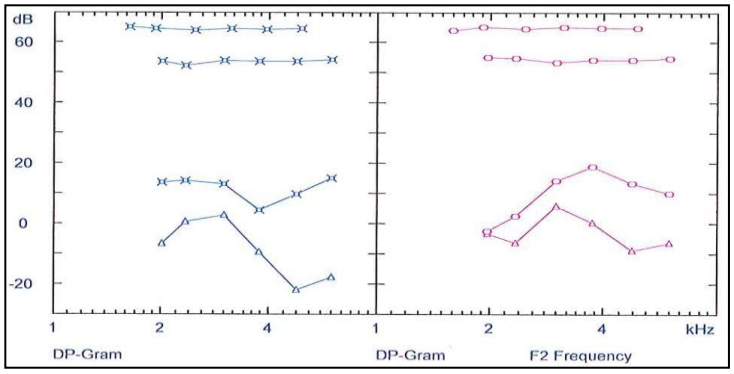
(e) Subject 6 – left and right ear



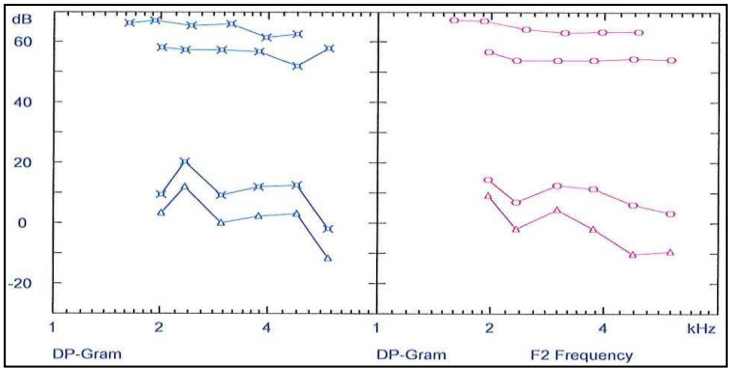
(f) Subject 7 – left and right ear



(g) Subject 8 – left and right ear



(h) Subject 9 – left and right ear



(i) Subject 10 – left and right ear

REFERENCES

Abdala, C., Oba, S., & Ramanathan, R. (2008). Changes in the DP-gram during the preterm and early postnatal period. *Ear & Hearing*; 29(4): 512-523. doi: 10.1097/AUD.0b013e31816c40bb.

Agrawal, V.K., Shukla, R., Misra, P.K., Kapoor, R.K., & Malik, G.K. (1998). Brainstem auditory evoked response in newborns with hyperbilirubinemia. *Indian Journal of Pediatrics*; 35(6): 513-8.

Ahdab-Barmada, M. & Moossy, J. (1984). The neuropathology of kernicterus in the premature neonate: diagnostic problems. *Journal of Neuropathology & Experimental Neurology*; 43: 45–56.

Ahlfors, C.E. (1994). Criteria for exchange transfusion in jaundiced newborns. *Pediatrics*; 93(3): 488-94.

Allen, F. H., Diamond, L. K., & Vaughan, V. C. (1950). Erythroblastosis fetalis. VI. Prevention of kernicterus. *American Medical Association American Journal of Diseases in Children*; 80(5): 779-791.

Allen, F. H., Diamond, L. K., & Watrous, J. B. (1949). Erythroblastosis fetalis; the value of blood from female donors for exchange transfusion. *New England Journal of Medicine*; 241(21): 799-806.

Amato, M. M., Kilguss, N. V., Gelardi, N. L., & Cashore, W. J. (1994). Dose-effect relationship of bilirubin on striatal synaptosomes in rats. *Biol Neonate*; 66: 288-93.

Amatuzzi, M., Northrop, C., Liberman, M., Thornton, A., Halpin, C., Herrmann, B., Pinto, L. E., Saenz, A., Carranza, A., Eavey, R. D. (2001). Selective inner hair cell loss in premature infants and cochlea pathological patterns from neonatal intensive care unit autopsies. *Achieves of Otolaryngology - Head & Neck Surgery*; 127: 629-636.

American Academy of Pediatrics. (1994). Practice parameters: management of hyperbilirubinemia in the healthy term newborn. *Pediatrics*; *94*: 558-565.

American Academy of Pediatrics. (2004). Clinical practice guidelines: management of hyperbilirubinemia in the newborn infant 35 or more weeks of gestation. *Pediatrics*; 114: 297-316.

American Academy of Pediatrics, Committee of Environmental Health. (1997). Noise: a hazard for the fetus and newborn. *Pediatrics*; 100(4): 724-727.

Amin, S. B. (2004). Clinical assessment of bilirubin-induced neurotoxicity in premature infants. *Seminars in Perinatology*; 28(5): 340-347. doi: 10.1053/j.semperi.2004.09.005

Amin, S. B., Ahlfors, C., Orlando, M. S., Dalzell, L. E., Merle, K. S., & Guillet, R. (2001). Bilirubin and serial auditory brainstem responses in premature infants. *Pediatrics*; 107: 664-670.

Amin, S. B., Orlando, M. S., Dalzell, L. E., Merle, K. S., & Guillet, R. (1999). Morphological changes in serial auditory brain stem responses in 24 to 32 weeks' gestational age infants during the first week of life. *Ear & Hearing*; 20: 410-418.

Arnold, S. (2000). The auditory brain stem response. In R. Roeser, M. Valente, & H. Hosford-Dunn (Ed.): *Audiology: Diagnosis*. New York, NY: Medical Publishers, Inc.; 451-470.

Ballachanda, B., Crawford, M., Ferraro, J., & Griffiths, S. (2003). Auditory evoked potentials. American Speech-Language-Hearing Association. Retrieved from the internet on February 16, 2007 from http://www.asha.org/NR/rdonlyres/CA4789BD-54B5-4A00-92E5-E4E4786CB30B/0/AEPtutorial.pdf .

Bergman, I., Hirsch, R. P., Fria, T. J., Shapiro, S. M., Holzman, I., & Painter, M. J. (1985). Cause of hearing loss in the high-risk premature infant. *The Journal of Pediatrics*; 106(1): 95-101.

Bhutani, V.K. & Johnson, L.H. (2004). Urgent clinical need for accurate and precise bilirubin measurements in the United States to prevent kernicterus. *Clinical Chemistry*; 50: 477-480.

Bhutani, V.K., Johnson, L.H., & Sivieri, E.M. (1999). Predictive ability of a predischarge hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. *Pediatrics*; 103: 6-14.

Billings, B.H., Cole, P.G., & Lathe, G.H. (1954). Increased plasma bilirubin in newborn infants in relation to birth weight. *British Medical Journal*; 2: 1263-5.

Billings, K.R. & Kenna, M.A. (1999). Causes of pediatric sensorineural hearing loss. *Archives of Otolaryngology-Head & Neck Surgery*; 125: 517-521.

Bratlid, D. (1990). How bilirubin gets into the brain. *Clinics in Perinatology*; 17: 449-65.

Brouillard, R. (1974). Measurement of red blood cell life-span. *Journal of the American Medical Association*; 230: 1304-1305.

Brown, D. K., Bowman, D. M., & Kimberley, B. P. (2000). The effects of maturation and stimulus parameters on the optimal f_2/f_1 ratio of the $2f_1$-f_2 distortion product otoacoustic emission in neonates. *Hearing Research*; 145: 17-24.

Buchman, C. A., Roush, P. A., Teagle, H. F., Brown, C. J., Zdanski, C. J., & Grose, J. H. (2006). Auditory neuropathy characteristics in children with cochlear nerve deficiency. *Ear & Hearing*; 27: 399-408.

Burkard, R. F. & Secor, C. (2002). Overview of auditory evoked potentials. In J. Katz (Ed.): *Handbook of Clinical Audiology*. 5th ed. Baltimore, Maryland: Lippincott Williams & Wilkins; 233-248.

Cashore, W. J. (2000). Bilirubin and jaundice in the micropremie. *Clinics in Perinatology*; 27: 171-179.

Cashore, W. J. & Oh, W. (1982). Unbound bilirubin and kernicterus in low birth weight infants. *Pediatrics*; 69: 481-485.

Chisin, R., Perlman, M., & Sohmer, H. (1979). Cochlear and brain stem responses in hearing loss following neonatal hyperbilirubinemia. *The Annals of Otology, Rhinology & Laryngology*; 88: 352-357.

Chuniaud, L., Dcssantc, M., Chantoux, F., Blondeau, J. P., Francon, J., & Trivin, E. (1996). Cytotoxicity of bilirubin for human fibroblasts and rat astrocytes in culture: effect of the ratio of bilirubin to serum albumin. *Clinica chimica acta; International Journal of Clinical Chemistry*; 256: 103-114.

Conlee, J. W. & Shapiro, S. M. (1991). Morphological changes in the cochlear nucleus and nucleus of the trapezoid body in Gunn rat pups. *Hearing Research*; 57(1): 23-30.

Cox, L. C. (1985). Infant assessment: developmental and age related considerations. In J. Jacobson (Ed): *The Auditory Brainstem Response*. San Diego: College-Hill Press.; 301.

Cremer, R. J., Perryman, P. W., & Richards, D. H. (1958). Influence of light on the hyperbilirunbinemia of infants. *Lancet*; 1: 1094-1017.

Darcy, A., Hancock, L., & Ware, E. (2008). A descriptive study of noise in the neonatal intensive care unit. *Advances in Neonatal Care*; 8(3): 165-175.

Deltenre, P., Mansbach, A. L., Bozet, C., Clercx, A., & Hecox, K. E. (1997). Auditory neuropathy: a report on three cases with early onsets and major neonatal illnesses. *Electroencephalography and clinical neurophysiology*; 104(1): 17-22.

Dennery, P.A., Seidman, D.S., and Stevenson, D.K. (2001). Neonatal hyperbilirubinemia. *The New England Journal of Medicine*; 344: 581-590.

de Vries, L. S., Lary, S., & Dubowitz, L. M. S. (1985). Relationship of serum bilirubin levels to ototoxicity and deafness in high-risk low birth weight infants. *Pediatrics*; 76: 351-354.

Diamond, L. K. (1948). Replacement transfusion as a treatment for erythroblastosis fetalis. *Pediatrics*; 2(5): 520-524.

Diamond, L. K., Allen, F. H. Jr., & Thomas, W. O. (1951). Erythroblastosis fetalis. VII. Treatment with exchange transfusion. *New England Journal of Medicine*; 244: 39-49.

Don, M. & Kwong, B. (2002). Auditory Brainstem Response: differential diagnosis. In J. Katz (Ed.): *Handbook of Clinical Audiology*. 5th ed. Baltimore, Maryland: Lippincott, Williams & Wilkins 274-297.

Dublin, W. (1951). Neurological lesions in erythroblastosis fetalis in relation to nuclear deafness. *American Journal of Clinical Pathology*; 21: 935–9.

Dublin, W. (1976). *Fundamentals of Sensorineural Auditory Pathology*. Springfield (IL): Charles C. Thomas.

Elberling, C., & Don, M. (1987). Threshold characteristics of the human auditory brain stem response. *Journal of the Acoustical Society of America*; 81: 115-121.

Ferraro, J. (1997). Laboratory exercises in auditory evoked potentials. San Diego, CA: Singular Publishing Group, Inc.

Funato, M., Tamai, H., Shimada, S., & Nakamura, H. (1994). Vigintiphobia, unbound bilirubin, and auditory brainstem responses. *Pediatrics*; 93: 50-53.

Garg, A. K., Prasad, R. S., & Hifzi, I. A. (1995). A controlled trial of high-intensity double-surface phototherapy on a fluid bed versus convertional phototherapy in neonatal jaundice. *Pediatrics*; 95: 914-916.

Gartner, L. M., Herrarias, C. T., & Sebring, R. H. (1998). Practice patterns in neonatal hyperbilirubinemia. *Pediatrics*; 101(1): 25-31.

Gartner, L. M., Snyder, R. N., Chaban, R. S., & Bernstein, J. (1970). Kernicterus: high incidence in premature infants with low serum bilirubin concentrations. *Pediatrics*; 45: 906-917.

Gerrard J. (1952). Nuclear jaundice and deafness. *Journal of Laryngology & Otology*; 66: 39-46.

Goldson, E. (1999). *Nurturing the Premature Infant: Developmental Interventions in the Neonatal Intensive Care Nursery.* New York: Oxford University Press.

Gorga, M. P., Kaminski, J. R., Beuachaine, K. L., & Bergman, B. M. (1993a). A comparison of auditory brain stem response thresholds and latencies elicited by air- and bone-conducted stimuli. *Ear & Hearing*; 14(2): 85-94.

Gorga, M. P., Neely, S. T., Bergman, B., Beauchaine, K. L., Kaminski, J. R., Peters, J., & Jesteadt, W. (1993b). Otoacoustic emissions from normal-hearing and hearing-impaired subjects: distortion product responses. *Journal of the Acoustical Society of America*; 93(4 Pt 1): 2050-2060.

Gorga, M. P., Neely, S. T., Ohlrich, B., Hoover, B., Redner, J., & Peters, J. (1997). From laboratory to clinic: a large scale study of distortion product otoacoustic emissions in ears with normal hearing and ears with hearing loss. *Ear & Hearing*; 18(6): 440-455.

Gorga, M. P., Norton, S. J., Sininger, Y. S., Cone-Wesson, B., Folsom, R. C., Vohr, B. R., Widen, J. E., & Neely, S. T. (2000). Identification of neonatal hearing impairment: distortion product otoacoustic emissions during the perinatal period. *Ear & Hearing*; 21(5): 400-424.

Gorga, M. P., Reiland, J. K., Beauchaine, K. A., Worthington, D. W., & Jesteadt, W. (1987). Auditory brainstem responses from graduates of an intensive care nursery: normal patterns of response. *Journal of Speech and Hearing Research*; 30: 311-318.

Grundmeier, R. W., Swietlik, M., & Bell, L. M. (2007). Research subject enrollment by primary care pediatricians using an electronic health record. *AMIA Annual Symposium Proceedings*. October 11: 289-293.

Hansen, T. W. (1996). Therapeutic approaches to neonatal jaundice. An international survey. *Clinical Pediatrics*; 35: 309-316.

Harris, R.C. (1961). Peak levels of serum bilirubin in normal premature infants. In A. Sass-Kortsak (Ed.): *Kernicterus*. Toronto, Canada: University of Toronto Press; 10-12.

Hass, P. (1999). Differentiation and diagnosis of jaundice. *American Association of Critical-Care Nurses Clinical Issues Advanced Practice in Acute Critical Care*; 10(4): 433-441.

Haymaker, W. Margles, C., & Pentschew, A. (1961). Pathology of kernicterus and posticteric encephalopathy. In C. A. Swinyard (ed.): *Kernicterus and Its Importance in Cerebral Palsy*. Springfield (IL): Charles C. Thomas: 21-229.

Hoffman, D. H., Zanelli, S. A., Kubin, J., Mishra, O. P., & Delivoria-Papadopoulos, M. (1996). The in vivo effect of bilirubin on the N-methyl-D-aspartate receptor/ ion channel complex in the brains of newborn piglets. *Pediatric Research*; 40: 804-808.

Hovi, L. & Siimes, M. A. (1985). Exchange transfusion with fresh heparinized blood is a safe procedure: Experiences from 1069 newborns. *Acta Paediatr Scand*; 74: 360-365.

Hulzebos, C.V., van Imhoff, D.E., Bos, A.F., Ahlfors, C.E., Verkade, H.J., & Dijk, P.H. (2008). Usefulness of bilirubin/albumin ratio for predicting bilirubin-induced neurotoxicity in premature infants. *Archives of Disease in Childhood-Fetal and Neonatal Edition*; doi:10.1136/adc.2007.134056.

Hyde, M. L. (1985). The effect of cochlear lesions on the ABR. In J.T. Jacobson (Ed.): *Auditory Brainstem Response*. San Diego, CA: College-Hill Press; 133-146.

Ives, N. K. (1999). Neonatal jaundice. In J. M. Rennie & N. R. C. Roberton (Eds.): *Textbook of neonatology.* New York: Churchill Livingston: 715-732.

Jackson, J. C. (1997). Adverse events associated with exchange transfusion in healthy and ill newborns. *Pediatrics*; 99(5):e7. Available at: www.pediatrics.org/cgi/content/full/99/5/e7

Jerger, J., & Mauldin, L. (1978). Prediction of sensorineural hearing level from the brain stem evoked response. *Archives of Otolaryngology*; 104: 456-461.

Johnson, R. & Kuby, P. (2004). *Elementary Statistics*. 9th ed. Belmont, CA: Brooks/Cole, Thompson Learning Inc.; 654-707.

Joint Committee on Infant Hearing (2007). Year 2007 Position Statement: Principles and guidelines for early hearing detection and intervention programs. *Pediatrics*; 120: 898-921.

Kaga, K., Kitazumi, E., & Kodama, K. (1979). Auditory brain stem responses of kernicterus infants. *International Journal of Pediatric Otorhinolaryngology*; 1: 255-264.

Kaplan, M., Muraca, M., Hammerman, C., Rubaltelli, F., Vilei, M. T., Vreman, H. J., & Stevenson, D. K. (2002). Imbalance between production and conjugation of bilirubin: a fundamental concept in the mechanism of neonatal jauncide. *Pediatrics*; 110(4): e47.

Keefe, D. H. & Abdala, C. (2007). Theory of forward and reverse middle-ear transmission applied to otoacoustic emissions in infants and adult ears. *Journal of the Acoustical Society of America*; 121(2): 978-993.

Keefe, D. H., Bulen, J. C., Arehart, K. H., & Burns, E. M. (1993). Ear-canal impedance and reflection coefficient in human infants and adults. *The Journal of the Acoustical Society of America*; 94(5): 2617-2638.

Keenan, W. J., Novak, K. K., Sutherland, J. M., Bryla, D. A., & Fetterly, K. L. (1985). Morbidity and mortality associated with exchange transfusion. *Pediatrics*; 75: 417-421.

Kelemen, G. (1956). Erythroblastosis fetalis. Pathologic report on the hearing organs of the newborn infant. *American Medical Association – Archives of Otolaryngology*; 63: 392-398.

Kemp, D. T. (1978). Stimulated acoustic emissions from within the human auditory system. *Journal of Acoustical Society of America*; 64: 1386-1391.

Kraus, N., Ozdamar, O., Stein, L., & Reed, N. (1984). Absent auditory brain stem response: peripheral hearing loss or brain stem dysfunction? *Laryngoscope*; 94:400-6.

Kreuger, C., Wall, S., Parker, L., & Nealis, R. (2005). Elevated sound levels within a busy NICU. *Neonatal Netw*; 24(6): 33-37.

Kroll, J. A. (editor). (2001). *An Industry in Evolution*. Boston, Mass: CenterWatch.

Lasky, R. & Williams, A. (2009). Noise and light exposures for extremely low birth weight newborns during their stay in the neonatal intensive care unit. *Pediatrics*; 123: 540-546.

Lauer, B. A., Githens, J. H., Hayward, A. R., Conrad, P. D., Yanagihara, R. T., & Tubergen, D. G. (1982). Probable graft-vs-graft reaction in an infant after exchange transfusions. *Pediatrics*; 70: 43-47.

Lightner, D. A. & McDonagh, A. F. (1984). Molecular mechanisms of phototherapy for neonatal jaundice. *Accounts of Chemical Research*; 17: 417-424.

Livaditis, A., Wallgren, G., & Faxeluis, G. (1974). Necrotizing enterocolitis after catheterization of the umbilical vessels. *Acta Paediatr Scand*; 63: 277-282.

Lonsbury-Martin, B. & Martin, G. (2007). In M. Robinette & T. Glattke (Eds.): *Otoacoustic Emissions: Clinical Applications*. 3rd ed. New York: Thieme Medical Publishers; 107-130.

Lucey, J., Ferriero, M., & Hewitt, J. (1968). Prevention of hyperbilirubinemia of prematurity by phototherapy. *Pediatrics*; 41: 1047-1054.

Madden, C., Rutter, M., Hilbert, L., Greinwald, J. H. Jr., & Choo, D. I. (2002). Clinical and audiological features in auditory neuropathy. *Archives of Otolaryngology-Head & Neck Surgery*; 128: 1026-1030.

Maisels, M. J. (2001). Neonatal hyperbilirubinemia. In M. H. Klaus & A. A. Fanaroff (Eds.): *Care of the High Risk Neonate*. Philadelphia: WB Saunders, Co.; 324-362.

Maisels, M. J. (2001). Phototherapy: traditional and nontraditional. *Journal of Perinatology*; 21: S93-S97.

Maisels, M. J. (1999). Jaundice. In G. B. Avery, M. A. Fletcher, & M. G. McDonald (Eds.): *Neonatology: pathophysiology and management of the newborn.* Philadelphia: JB Lippincott, Co; 765-819.

Maisels, M. J. (1996). Why use homeopathic doses of phototherapy? *Pediatrics*; 98: 283-287.

Maisels, M. J. & Kring, E. (2006). The contribution of hemolysis to early jaundice in normal newborns. *Pediatrics*; 118: 276-279.

Maisels, M. J. & McDonagh, A. F. (2008). Phototherapy for neonatal jaundice. *New England Journal of Medicine*; 358: 920-928.

Maisels, M. J. & Newman, T. B. (1995). Kernicterus in otherwise healthy, breast-fed term newborns. *Pediatrics*; 96: 730-733.

Maisels, M.J. & Watchko, J.F. (2003). Treatment of jaundice in low birthweight infants. *Archives of Disease in Childhood-Fetal and Neonatal Edition*; 88(6): F459-F463.

Martinez, J. C., & Garcia, H. O., Otheguy, L. E., Drummond, G. S., & Kappas, A. (2001). Treatment of hyperbilirubinemia pharmacologic approach SnMP (tin-mesoporphyrin). *Journal of Perinatology*; 21: S101-S103.

Matkin, N. D. & Carhart, R. (1966). Auditory profiles associated with Rh incompatibility. *Archives of Otolaryngology*; 84: 56-67.

Mosis, G., Dieleman, J. P., Stricker, B. Ch., van der Lei, J., & Sturkenboom, M. C. (2006). A randomized database study in general practice yielded quality data but patient recruitment in routine consultation was not practical. *Journal of Clinical Epidemiology*, 59(5): 497-502.

Møller, A. R. (1994). Neural generators of auditory evoked potentials. In J. Jacobson(Ed.): *Principles and Applications in Auditory Evoked Potentials*. Boston: Allyn & Bacon; 23-46.

Møller, A. R., Jannnetta, P. J., & Jho, H. D. (1994). Click-evoked responses from the cochlear nucleus: a study in human. *Electroencephalography and clinical neurophysiology*; 92(3): 215-224.

Møller, A. R., Jho, H. D., Yokota, M., & Jannetta, P. J. (1995). Contribution from crossed and uncrossed brainstem structures to the brainstem auditory evoked potentials: a study in humans. *Laryngoscope*; 105(6): 596-605.

Musiek, F. (2010, January 19). Hyperbilirubinemia and auditory neuropathy/auditory dys-synchrony: interview with Frank Musiek, PhD. Retrieved February 3, 2010, from American Academy of Audiology website: http://www.audiology.org/news/Pages/20100119.aspx

Nakamura, H., Takada, S., Shimabuku, R., Matsuo, M., Matsuo, T., & Negishi, H. (1985). Auditory nerve and brainstem responses in newborn infants with hyperbilirubinemia. *Pediatrics*; 75(4): 703-708.

National Institutes of Health (NIH). (1993). *Consensus statement: early identification of hearing impairment in infants and young children*. Online Mar 1-3; 11(1): 1-24.

Newman, T. B. & Maisels, M. J. (1992). Evaluation and treatment of jaundice in the term newborn: a kinder, gentler approach. *Pediatrics*; 89: 809-818.

Norton, S. J., Widen, J. E., Gorga, M. P., Folsom, R. C., Sininger, Y., Cone-Wesson, B., Vohr, B. R., Mascher, K., & Fletcher, K. (2000). Identification of neonatal hearing impairment evaluation of transient evoked otoacoustic emission, distortion product otoacoustic emission, and auditory brain stem response test performance. *Ear & Hearing*; 21(5): 508-528.

Ogun, B., Serbetcioglu, B., Duman, N., Ozkan, H., & Kirkim, G. (2003). Long-term outcomes of neonatal hyperbilirubinemia: subjective and objective audiological measures. *Clinical Otolaryngology*; 28: 507-13.

O'Shea, T. M., Dillard, R. G., Klinepeter, K. D., & Goldstein, D. J. (1992). Serum bilirubin levels, intracranial hemorrhage, and the risk of developmental problems in very low birth weight infants. *Pediatrics*; 90: 888-892.

Oysu, C., Ulubil, A., Aslan, I., & Baserer, N. (2002). Incidence of cochlear involvement in hyperbilirubinemic deafness. *The Annals of Otology, Rhinology & Laryngology*; 111: 1021-1025.

Parimi, P. (2008, December 3). Associate Professor of Neonatology, University of Kansas Medical Center. Personal communication.

Perlman, M., Fainmesser, P., Sohmer, H., Tamari, H., Wax, Y., & Pevsmer, B. (1983). Auditory nerve-brainstem evoked responses in hyperbilirubinemic neonates. *Pediatrics*; 72(5): 658-664.

Prieve, B. A. & Fitzgerald, T. S. (2002). Otoacoustic Emissions. In J. Katz (Ed.): *Handbook of Clinical Audiology*. 5th ed. Baltimore: Baltimore, Maryland: Lippincott Williams & Wilkins; 440-466.

Rance, G., Beer, D. E., Cone-Wesson, B., Shepherd, R. K., Dowell, R. C., King, A. M., Rickards, F. W., & Clark, G. M. (1999). Clinical findings for a group of infants and young children with auditory neuropathy. *Ear & Hearing*; 20(3): 238-252.

Rapin, I. & Gravel, J. (2006). Auditory neuropathy: a biologically inappropriate label unless acoustic nerve involvement is documented. *Journal of American Academy of Audiology*; 17: 147-150.

Reddy, P., Najundaswamy, S., Mehta, R., Petrova, A., & Hegyi, T. (2003). Tin-mesoporphyrin in the treatment of severe hyperbilirubinemia in a very-low-birth-weight infant. *Journal of Perinatology*; 23: 507-508. DOI: 10.1038/sj.jp7210943

Rhee, C., Park, H., & Jang, Y. (1999). Audiologic evaluation of neonates with severe hyperbilirubinemia using transiently evoked otoacoustic emissions and auditory brainstem responses. *The Laryngoscope.*; 109(12): 2005-2008.

Robinette, M. S. (1992). Clinical observations with transient evoked otoacoustic emissions with adults. *Seminars in Hearing*; 13: 23-36.

Rotteveel, J. J., de Graaf, R., Colon, E. J., Stegeman, D. F., & Visco, Y. M. (1987). The maturation of the central auditory conduction in preterm infants until three months post term. II. The auditory brainstem responses (ABRs). *Hearing Research*; 26: 21-35.

Seidman, D. S., Moise, J, Ergaz, Z., Laor, A., Vreman, H. J., Stevenson, D. K., & Gale, R. (2000). A new blue light-emitting phototherapy device: a prospective randomized controlled study. *Journal of Pediatrics*; 136: 771-774.

Shapiro, S. M. (1993). Reversible brainstem auditory evoked potential abnormalities in jaundiced Gunn rats given sulfonamide. *Pediatric Research*; 34(5): 629-633.

Shapiro, S. M. & Conlee, J. W. (1991). Brainstem auditory evoked potentials correlate with morphological changes in Gunn rat pups. *Hearing Research*; 57(1): 16-22.

Shapiro, S. M. & Daymond, M. J. (2003). Patterns of kernicterus related to neonatal hyperbilirubinemia and gestational age. *Pediatric Research*; 53-54(Part 2): 398A-399A.

Shapiro, S. M. & Hecox, K. E. (1988). Developmental studies of brainstem auditory evoked potentials in jaundiced Gunn rats. *Brain Research*; 469(1-2): 147-157.

Shapiro, S. M. & Hecox, K. E. (1989). Brain stem auditory evoked potentials in jaundiced Gunn rats. *Annals of Otology, Rhinology & Laryngology*; 98(4 Pt 1): 308-317.

Shapiro, S. M. & Nakamura, H. (2001). Bilirubin and the auditory system. *Journal of Perinatology*; 21: S52-S55.

Shapiro, S. M., Rosen, J. R., & Dixon, K. T. (2002). Auditory brainstem responses and auditory neuropathy abnormalities in children with neonatal hyperbilirubinemia who subsequently develop kernicterus. *Pediatric Research*; 51(Part 2): 340A

Sheykholeslami, K. & Kaga, K. (2000). Otoacoustic emissions and auditory brainstem responses after neonatal hyperbilirubinemia. *International Journal of Pediatric Otorhinolaryngology*; 52(1):65-73.

Sininger, Y. (1993). Auditory brain stem response for objective measures of hearing. *Ear & Hearing*; 14: 23-30.

Sininger, Y. (1995). Filtering and spectral characteristics of averaged auditory brain-stem response and background noise in infants. *Journal of the Acoustical Society of America*; 98(4): 2048-2055.

Sininger, Y. & Cone-Wesson, B. (2002). Threshold prediction using auditory brainstem response and steady-state evoked potentials with infants and young children. In J. Katz (Ed.): *Handbook of Clinical Audiology*. 5th ed. Baltimore, Maryland: Lippincott, Williams & Wilkins; 298-322.

Smurzynski, J. (1994). Longitudinal measurements of distortion-producet and click-evoked Otoacoustic emissions of preterm infants: preliminary results. *Ear & Hearing*; 15(3): 210-223.

Smurzynski, J., Jung, M., Lafreniere, D., Kim, D., Kamath, M.V., Rowe, J., Holman, M., & Leonard, G. (1993). Distortion-product and click-evoked otoacoustic emissions of preterm and full-term infants. *Ear & Hearing*; 14(4): 258-274.

Stegeman, D. F., Van Oosterom, A., Colon, E. J. (1987). Far-field evoked potential components induced by a propagating generator: computational evidence. *Electroencephalogram Clinical Neurophysiology*; 67: 176-187.

Stein, L.K., Tremblay, K., Pasternak, J., Banerjee, S., Lindemann, K., & Kraus, N. (1996). Brainstem abnormalities in neonates with normal otoacoustic emissions. *Seminars in Hearing*. 17:2, 197-213.

Stevens, J. C. (1988). Click-evoked oto-acoustic emissions in normal and hearing-impaired adults. *British Journal of Audiology*; 2: 45-49.

Suresh, G., Martin, C. L., & Soll, R. (2003). Metalloporphyrins for treatment of unconjugated hyperbilirubinemia in neonates. *Cochrane Database Systematic Reviews*; 1: CD004207. doi: 10.1002/14651858.CD004207

Tan, K. L. (1991). Phototherapy for neonatal jaundice. *Clinical Perinatology*; 18: 423-439.

Tan, K. L., Skurr, B. A., & Yip, Y. Y. (1992). Phototherapy and the brainstem auditory evoked response in neonatal hyperbilirubinemia. *Journal of Pediatrics*; 120: 306-308.

Turkel, S. B., Miller, C. A., Guttenberg, M. E., Moynes, D. R., & Hodgman, J. E. (1982). A clinical pathologic reappraisal of kernicterus. *Pediatrics*; 69: 267-272.

van de Bor, M., van Zeben-van der Aa, T. M., Verloove-Vanhorick, S. P., Brand, R., & Ruys, J. H. (1989). Hyperbilirubinemia in preterm infants and neurodevelopmental outcome at two years of age: results of a national collaborative survey. *Pediatrics*; 83: 915-920.

Vreman, H. J., Wong, R. J., Stevenson, D. K., Route, R. K., Reader, S. D., Fejer, M. M., Gale, R., & Seidman, D.S. (1998). Light-emitting diodes: a novel fight source for phototherapy. *Pediatric Research*; 44:804-809.

Vohr, B.R., Widen, J.E., Cone-Wesson, B., Sininger, Y.S., Gorga, M.P., Folsom, R.C., & Norton, S.J. (2000). Identification of neonatal hearing impairment: characteristics of infants in the neonatal intensive care unit and well-baby nursery. *Ear & Hearing*; 21(5): 373-382.

Vohr, B. R., Karp, D., O'Dea, C., Darrow, D., Coll, C. G., Lester, B. M., Brown, L, Oh, W., & Cashore, W. (1990). Behavioral changes correlated with brain-stem auditory evoked responses in term infants with moderate hyperbilirubinemia. *Journal of Pediatrics*; 117(2 Pt 1): 288-291.

Walsh, W., McCullough, K., & White, R. (2006). Room for improvement: nurses' perceptions of providing care in a single room newborn intensive care setting. *Advances in Neonatal Care*; 6: 261-270.

Watchko, J.F. (2000). The clinical sequelae of hyperbilirubinemia. In M. J. Maisels & J. F. Watchko, (eds.): *Neonatal Jaundice.* Amsterdam: Harwood Academic Publishers; 115-135.

Watchko, J. F. (2000). Exchange transfusion in the management of neonatal hyperbilirubinemia. In M. J. Maisels & J. F. Watchko (eds.): *Neonatal Jaundice*. Amsterdam: Harwood Academic Publishers; 169-176.

Watchko, J. F. & Maisels, M. J. (2003). Jaundice in low birthweight infants: pathobiology and outcome. *Archives of Disease in Childhood-Fetal and Neonatal Edition*; 88:F455-F458.

Wennberg, R. P., Ahlfors, C. E., Bhutani, V. K., Johnson, L. H., & Shapiro, S. M. (2006). Toward understanding kernicterus: a challenge to improve the management of jaundiced newborns. *Pediatrics*; 117(2): 474-485. doi: 10.1542/peds.2005-0395

Wong, V., Chen, W. X., & Wong, K. Y. (2005). Short- and long-term outcome of severe neonatal nonhemolytic hyperbilirubinemia. *Journal of Child Neurology*; 21: 309-315.